A Summary of Current Program, 7/1/64;

and Preliminary Report of Progress

for 7/1/63 to 6/30/64

NORTHERN

UTILIZATION RESEARCH AND DEVELOPMENT

DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1963, and June 30, 1964. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE

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TABLE OF CONTENTS

INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of four research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for utilization research concerned with industrial utilization of cereal grains, soybeans and flaxseed and with food and feed uses of corn, sorghum and soybeans. Responsibility for food and feed uses of wheat is assigned to the Western Division. In the Department's program of research on replacement crops the Northern Division conducts all screening and characterization studies on uncultivated plants and their components and is responsible for more intensive utilization research on new oilseeds containing erucic acid and on new gum and pulp fiber plants. Responsibility for detailed studies of additional selected new oilseeds is divided among the other three utilization research divisions. The Northern Division also maintains small programs on sugarcane and forages. The major part of the Department's utilization research on sugarcane and forages is conducted at the Southern and Western Divisions, respectively.

In this report, utilization research of the Northern Division is discussed under the 11 Area Headings shown in the Table of Contents. For each area, a description of the current research program is provided, including domestic research contracts and grants and sponsorship of related research performed abroad under grants of Public Law 480 funds. A preliminary report of progress and a list of publications is given for each area for the period July 1, 1963, through June 30, 1964. A description of related work of State Experiment Stations is also provided for each area.

The scientific research effort at the Northern Division amounts to approximately 235 professional man-years. In addition, the Division supervises domestic research contracts equivalent to 40.0 professional man-years and grants equivalent to 4.6 professional man-years, and sponsors a comprehensive program of research comprising 50 PL 480 grants. Following are some of the recent utilization research accomplishments of the Northern Division.

Dialdehyde starch improves plywood glues. The Department's research has produced a superior glue for interior grade plywood for the developing southern pine plywood industry in which the conventional soyflour-based protein-type glues used in Douglas fir plywood have proven unsatisfactory. The Douglas fir plywood industry, which in the past supplied almost all of the softwood plywood, consumed over 150 million pounds of protein glues in 1963. The softwood plywood production has been increasing about 10 percent per year and future growth will probably come largely from southern pine. The first commercial production of southern pine plywood was in 1963. Addition of the Department-developed dialdehyde starch to conventional protein glues has given good performance with southern pine interior plywoods at about one-half the cost of synthetic glues that have been used to date.

Linseed oil for curing concrete. Based on results of cooperative work of the Department with the National Flaxseed Processors Association, linseed oil is finding a constantly increasing use for protecting cured concrete against freeze-thaw deterioration. Currently, research by Department scientists has shown that linseed oil emulsions have considerable promise for spray-type compounds to prevent moisture losses in the curing of concrete. In order to develop optimum strength properties, freshly laid concrete must be protected while curing against loss of water through surface evaporation. Presently this protection is obtained by solid coverings such as polyethylene sheeting or by spraying on an impervious surface coating. Research now underway indicates that the linseed oil applied to freshly laid concrete to aid in curing also acts as a freeze-thaw protective agent, thus giving a dual benefit from its use. The linseed oil is applied as a water emulsion using conventional road-spraying equipment. This development has a potentiality for a new multimillion-pound market for linseed oil.

New fermentation process developed for production of 2-ketogluconic acid from corn sugar. The Department has developed a new fermentative organism and an improved process for manufacturing from corn sugar the industrial chemical, 2-ketogluconic acid. Yields are higher and less time is required for the new procedure than by previous processes. A number of companies have made inquiry about the new process and several have requested cultures for making pilot runs. Several million pounds of 2-ketogluconic acid are used annually in the production of isoascorbic acid, a material used as an antioxidant in the preparation of various food products including meat. This more efficient method for making the 2-ketogluconic acid will assure still greater usage of this cereal-derived product.

Crambe, a promising new crop, successfully processed on commercial scale. Department research studies on processing crambe seed to oil and meal made possible a successful 40-ton commercial-plant run. Only minor equipment modifications in a conventional oilseed plant were required to obtain good recovery of oil and meal. Adequate supplies of crambe oil and meal products

are now available for industrial evaluations, animal-feeding studies, and continuing Department investigations. Because of the favorable results achieved in this processing demonstration, an industrial company has decided to grow several hundred acres of crambe during the 1964 season. Crambe, a plant related to rape and mustard, can be grown in many parts of the country and appears especially suitable as a replacement for crops now in over-supply. Crambe oil would compete with imported rapeseed oil, but not with presently grown domestic vegetable oils. One of the immediate uses for crambe oil is to utilize the erucic acid, the principal fatty acid of crambe oil, in the manufacture of dibasic brassylic acid which already has several industrial end-uses.

Improved procedure discovered for grafting acrylonitrile to starch. By an improved procedure devised by scientists at Stanford Research Institute under contract to the Department, acrylonitrile can be grafted rapidly to corn starch to give new physical and chemical properties to the starch. These acrylonitrile-starch graft copolymers can be converted readily to water-soluble products having potential as internal sizing agents and pigment retention aids for paper, drilling mud additives, and industrial adhesives. Graft polymerization is a promising new route to chemical modification of starch and development of starch-based products that can maintain competitive status and expand utilization of this agricultural product in today's market.

Pretempering improves processing of old or low-moisture corn. Department research has confirmed beneficial effects of pretempering in dry milling of old or low-moisture corn. In the pretempering step, which requires from 10 to 20 hours, moisture in the corn is brought up to a level of about 15-1/2 percent. The pretempered corn is further tempered and then milled by conventional procedures. Compared to the results obtained when the corn is tempered to the final moisture in one step, both total yield of grits for manufacture of prime goods and yield of flaking grits are increased. Recovery of oil is not significantly altered. The principal disadvantage is that degerminator throughput is decreased. Several corn dry millers are using pretempering and find that it is an inexpensive means of overcoming difficulties encountered with corn that has been stored for long periods.

AREA NO. 1: CEREAL STARCHES
INDUSTRIAL UTILIZATION

Problem. Starch accounts for about two-thirds the weight of all grains. Finding new, large-volume outlets for starch would, therefore, result in substantially increased consumption of cereal grains. Of the 5.6 billion pounds of cereal starch now produced, about 3.5 billion pounds is used ultimately in food products, and increases would be expected to follow population growth. However, the remaining 2.1 billion pounds find industrial outlets that offer opportunities for increases at a rate greater than that of population growth. Because starch must compete with products derived from nonagricultural sources, these opportunities can best be realized by a program of research designed both to maintain the competitive position of starch in its current uses and to develop economical new industrial uses.

That some success is being achieved in finding expanded uses for starch is evidenced by the fact that starch usage increased at a rate twice as fast as population growth over the past 10 years. Also, the rate of starch usage as a paper additive increased by 50 percent over the past 15 years.

Substantial new outlets for cereal starches and flours can be envisioned if basic research and development on several types of chemical and physical modification of starch and flour now in the experimental stage or anticipated can be prosecuted to successful conclusion. This research is mainly directed toward new products for the pulp and paper industries and for the building and construction industries, but other industries, such as the chemical, petroleum,-mining, textile, plastics, coatings, and packaging industries, also provide attractive opportunities. New outlets for starch that appear very promising include use of modified starches as wet-strength additives for paper, water-resistant adhesives, coatings, and foamed products, and of starch-derived pulps as an integral part of high-quality paper. In addition, if the competitive position of starch is successfully maintained through improvement by research, additional consumption would be expected by 1975 from participation in the multimillion-bushel markets for grain resulting from normal growth of existing industrial outlets for starch and flour such as paper, textiles, packaging adhesives, drilling muds, and building materials.

To accelerate realization of these goals, more information is needed on the physical and chemical properties and chemical reactions of cereal starches, on economical methods for effecting desired physical and chemical modifications and on product evaluation and development. In addition, still further new markets for cereal starches should be possible from an adequate program of fundamental and exploratory research to discover new concepts, principles, and reactions leading to new processes and products for future development.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic and physical chemists and chemical engineers engaged in basic, applied and developmental studies on the chemistry of cereal starches and their conversion to useful industrial products.

The Federal scientific effort for research on cereal starches totals 52.0 professional man-years. Of this number 16.3 are devoted to chemical composition and physical properties and 35.7 to new starch chemical derivatives and their evaluation.

Research at Peoria, Illinois, on chemical composition and physical properties (11.5 professional man-years) involves fundamental research on reactions of maltose and glucose, on amino acid and peptide derivatives of carbohydrates, and principles of graft polymerization. Research contracts under this subheading (2.6 professional man-years) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies on the reaction of starch with mercaptans (.6 professional man-year) and with acetylene (.5 professional man-year) and on the reaction of acetylene with methyl glucoside (.6 professional man-year); with The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media (.7 professional man-year); and with Southern Illinois University, Carbondale, Illinois, for investigations on synthesis of maltooligosaccharides (.2 professional man-year). Grants (2.2 professional man-years) have been made to Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates (1.4 professional man-years) and to Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates (.8 professional man-year).

Research at Peoria, Illinois, on new starch chemical derivatives and their evaluation (21.3 professional man-years) involves basic and applied studies on various types of chemical products derived from starch and dextrin and in evaluation of these products for various industrial uses such as pulp and paper products, plastics, coatings, organic chemicals and stable viscosity agents. During the reporting period research was discontinued on the preparation and study of acetal and ketal derivatives of starch. Research contracts under this subheading (13.4 professional man-years) are in effect with the University of Minnesota, St. Paul, Minnesota, for studies on reactions of dialdehyde starch in solution (.4 professional man-year); with Ohio State University, Columbus, Ohio, for research on synthesis of amino derivatives of starch (1.2 professional man-years); with Battelle Memorial Institute, Columbus, Ohio, for developmental research on starch and other cereal grain xanthides (6.2 professional man-years); with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers

(1.3 professional man-years) and on process development of selected graft copolymers (2.3 professional man-years); and with Archer Daniels Midland Company, Minneapolis, Minnesota, for evaluation of starch polyol urethane foams (2.0 professional man-years). During the reporting period the following contract research was completed: studies on crosslinked hypochlorite oxidized starch in paper at the State University of New York, Syracuse, New York; and evaluation of allyl dialdehyde starch coatings at Battelle Memorial Institute, Columbus, Ohio.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the University of Birmingham, Birmingham, England, for research on starch structure as revealed by interaction of starch and enzymes (5 years, 1959-1964); National Institute of Hygiene, Paris, France, for research on proteolysis inhibiting effects of cereal starches and flours (3 years, 1961-1964); National Institute of Agronomic Research, Paris, France, for research on changes induced in starch by gamma irradiation (4 years, 1961-1965); Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (5 years, 1962-1967); Institute for Fibres and Forest Products, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968); and Lister Institute for Preventive Medicine, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968). New starch chemical derivatives and their evaluation involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); Institute of Industrial Chemistry, Bologna, Italy, for studies on fatty chemical derivatives of starch dextrins (5 years, 1960-1965); National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5 years, 1962-1967); Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968); and Academy of Sciences and Chemical Institute "Boris Kidric," Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969). During the reporting period research was completed on hypochlorite oxidation of starch at the Institute for Fibres and Forest Products, Jerusalem, Israel; on glucose-derived polymers at the Arthur D. Little Research Institute, Musselburgh, Scotland; and on fluorine derivatives of starch at Hebrew University, Jerusalem, Israel.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct a continuing program of research on the fundamental chemistry of cereal starches and their utilization. One regional project, NC-60, is directed to modification of starch for industrial uses. Participating States are seeking to: determine the fundamental reactions in the

nonenzymatic dextrinization of starch; investigate chemical polymerization of D-glucose derivatives for the production of new types of synthetic polymers; determine the mode of action of oxidants on starch; modify the basic structure of the D-glucose units in starch; discover enzymatic reactions which can modify starch and the effect of structural characteristics of starch on the action of enzymes; and develop methods by which nitrogen can be chemically attached to starch.

Other basic research is directed to study of the fundamental structure of complex carbohydrates and the mechanism of their formation and breakdown. Starch granules are being treated with enzymes, salt solutions, specific solvents and chemical reactants to determine in greater detail the microstructure and reactivity of starch granules. A statistical study of the reaction conditions for vinylation of starch with acetylene has been completed and evaluation of the reaction products with respect to their chemical and physical properties is now in progress. In another study, production of new types of hydrophilic polymers is being investigated by modification of polysaccharides by replacement of hydroxyl groups with mercapto, amino and by anhydro bridges and characterizing the physical and biochemical properties of the resulting polymers.

Enzyme systems from plants and bacteria are being examined from the point of view of their effect and role in structural changes, biosynthesis and deposition of starch. Radiation and chemical treatments are used in studies on the production of D-fructose from tubers of Jerusalem artichoke. A number of pure carbohydrases that will attack the whole spectrum of polysaccharide structure are being sought and their mechanism and specificity of action determined.

The total station scientific effort devoted to industrial utilization of starch is about 7.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Reactions of starch and dextrose in nonaqueous solvents. Research on nonaqueous reactions of starch and dextrose was completed. In final phases of the work, it was shown that maltose in liquid ammonia-ammonium chloride did not ammonolyze significantly to 1-amino glucose but that 1-amino maltose and dimaltosyl amine were formed. An infrared spectrophotometric method was developed for measuring the concentration of hydroxide ion in the simultaneous presence of water and ethanol.

This project has been highly productive of new basic information. The work has resulted in synthesis of a number of new carbohydrate derivatives, some of which, such as disorbitylamine, are of potential industrial interest. In addition, the work uncovered new principles of carbohydrate chemistry such as those influencing the formation of metal-carbohydrate adducts and/or

salts and those leading to insoluble high-molecular-weight amine derivatives (di-"starchosyl" amines) in ammonolysis of corn starch.

Research is being continued under a new project with the objective of discovering still further new reactions of dextrose and maltose. So far, a new, labile "diacetamide" derivative of maltose has been obtained by deacetylation of 1-aminomaltose octaacetate in methanol-ammonia.

2. Amino acid and peptide derivatives of starch. In exploratory studies on amino acid derivatives of starch, the reaction of glucose and N-carboxyglycine anhydride yielded an apparently stable glycosyl polyglycinate derivative. However, dialysis experiments in 60 percent aqueous lithium bromide showed that glucose could be removed from such products leaving polyglycine. Thus either the "product" was merely glucose dispersed in solid polyglycine, or any chemical binding between glucose and polyglycine was extremely labile and hydrolyzed by contact with aqueous lithium bromide.

3. Reactions of starch with acetylene and mercaptans. At the University of Arizona, monosubstituted glucose units were found to predominate in vinylated amylose and amylopectin when the degree of substitution was 1. In the monosubstituted units, vinyl groups occurred about equally at positions 2 and 6. Study of possible transvinylation between vinyl starch and a simple alcohol as acceptor showed that the reaction either did not occur or resulted merely in intramolecular migration of vinyl groups within vinyl starch. Preliminary results of a comparison of the reactions of acetylene with starch and cellulose revealed significant differences in the response of the reactions to variations in reaction conditions.

In other contract research at Arizona, promising products in terms of viscosity and light color have been obtained by condensing glucose and 1,10-decanedithiol. Statistically designed experiments are in progress to elucidate this reaction.

4. Reactions of starch in fluid dynamic media. At The Johns Hopkins University, a reactor, based on fluidized bed operation, for transforming starch to levoglucosan has been designed and is under construction.

5. Starch structure. At the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, where studies on the glucopyranose ring structure of starch are in progress under a PL 480 grant, significant data have been obtained indicating that the C_1 chair conformation is the probable form for glucopyranose rings in amylose, maltosaccharides, and linear and cyclic dextrins prepared from amylose. Data on the mode of interaction of dimethylsulfoxide (DMSO) with glucopyranosides suggest that the ring conformation in DMSO solution is the same or nearly the same as in the solid amorphous phase.

Scientists at the University of Birmingham, Birmingham, England, under a PL 480 grant, have developed a computer program to evaluate data obtained

in studies on interactions between starch and protein molecules, which profoundly affect the viscosity and solution properties of starches so important to their industrial uses. Concurrently, they have synthesized starch molecules of uniform size which are required as model compounds for their studies. These basic studies on starch-protein interactions are yielding information needed for improving the manufacture of starch from grain and for converting the isolated starch to more useful industrial products.

Studies are being initiated, under a PL 480 grant to the Lister Institute of Preventive Medicine, London, England, on starch- and glycogen-debranching enzymes and their application in study of the structure of cereal starches and components.

6. Proteolysis inhibition by starch. Comparative studies on the trypsin inhibitor activity of different cereal flours have continued with emphasis on stability, mode of action, effect on proteolysis pattern, and comparison of soybean trypsin inhibitor. Differences in stability of the trypsin inhibitor activity toward cooking and toward pepsin were observed in comparisons of flour from wheat, rice, millet, oats, corn, barley, rye, and buckwheat. Wheat bread showed no trypsin inhibiting activity but some was retained in rye bread. Malted barley retained activity. The incubation of corn at pH 12 destroyed antitrypsin activity. The mode of action of the inhibitor appeared to be similar for all of the cereals and to involve a strong bonding of the inhibitor to its site of action. The highest level of inhibitor was present in buckwheat flour but the amount was still relatively low since soybeans contained about 15 times as high a concentration of inhibitor. Studies on the isolation of trypsin inhibitor from wheat resulted in a procedure for concentrating the activity and a demonstration that two electrophoretic components, presumably peptides, have inhibitor activity.

This research is being performed by the National Institute of Hygiene, Paris, France, under a PL 480 grant.

7. Effects of gamma-radiation on starch. Scientists at the National Institute of Agronomic Research, Paris, France, under a PL 480 grant, have made good progress in an exhaustive study of the radiation-induced alterations of the starch molecule and the starch granule. Under the experimental conditions used, acidic groups and oligo- and higher saccharides were formed, and glucose units were degraded. The information resulting from this work not only contributes to basic knowledge of the chemistry of starch but also has specific significance in the research on starch graft copolymers (see following Part B, Item 3).

B. New Starch Chemical Derivatives and Their Evaluation

1. Studies on starch xanthates and xanthides. Amount of oxidant (Chlorox) required to crosslink cereal xanthate was found to be directly related to the amount of contained byproducts, such as sodium thiocarbonate, which are present in crude xanthate and increase with age. Freshly purified xanthate therefore required the least oxidant. Stability of a number of model xanthides was investigated and found to decrease as pH increases. Basic salts such as sodium sulfide and sodium sulfite had a greater destabilizing effect than could be attributed to pH alone, but neutral salts such as sodium chloride and sodium sulfate had no significant effect. Improved conditions were developed for crosslinking with zinc ion. Discovery that alum would function as a coprecipitating agent was mainly responsible for reduction of the zinc ion requirement to one-eighth of that formerly believed necessary.

Further studies to compare sodium hypochlorite and hydrogen peroxide in ex situ conversion of xanthate to xanthide showed that peroxide required a much higher xanthate group concentration than did hypochlorite to achieve quantitative conversion. The minimum concentration required by peroxide corresponds to a 4 percent solution of a D.S. 0.10 xanthate, whereas for hypochlorite only a 0.48 percent solution of D.S. 0.10 xanthate would be needed. The peroxide-converted products contained more sulfur, but had fewer intermolecular crosslinks. The high xanthate concentration required for crosslinking with hydrogen peroxide appears to rule out the use of peroxide for in situ incorporation of xanthides into paper unless partial crosslinking prior to dilution in the pulp furnish proves to be feasible.

At Battelle Memorial Institute optical and electron microscopic studies showed that in situ, in contrast to ex situ, crosslinking resulted in penetration of the fibrillar structure of paper and improved distribution of xanthide throughout the paper. A positive correlation between high strength of paper and good xanthide distribution was found. All needed analytical methods have been checked out and several new rapid methods have been developed.

These results show that good progress is being made in both in-house and contract research on the xanthate-xanthide process. Knowledge of the influence of byproducts initially present or formed on aging on the oxidant requirement for crosslinking xanthates is especially significant. Development of methods for reducing or eliminating easily oxidized byproducts and, if possible, for stabilizing xanthate solutions is indicated because of the obvious relationship to process economics. Demonstration of the relationship between proper strength and xanthide dispersion in paper provides an objective method for determining the effectiveness of various techniques for incorporating xanthides into paper. It also points to a possible interpretation of conflicting results obtained when ex situ crosslinked xanthides were used in paper and suggests possible advantages to development of finely

divided or colloidal xanthides. Since many prospective users of xanthides, particularly the smaller concerns, may be unable or unwilling to undertake xanthate preparation, the potential market would be significantly enlarged if an effective ex situ crosslinked xanthide were available.

2. Starch polyol foams. Basic characterization of rigid urethane foams from starch polyol glycosides has been completed. The data showed that glycoside-based polyethers with hydroxyl numbers of 407-445 yield foams comparable to those obtained from sorbitol polyethers of hydroxyl numbers of about 500. Because of the lower hydroxyl number of the starch-derived polyether, less diisocyanate is needed to produce a foam. Polyol glycoside polyethers with adequately uniform hydroxyl numbers and viscosities have been prepared. The "one-shot" technique was successfully used to produce rigid foams having good cell structure and physical properties (parallel compressive strength, 35-41 lbs./sq. in.; density, 1.9-2.0 lbs./cu. ft.).

These results continue to indicate considerable commercial promise for starch polyol foams. Indeed, following a recent presentation on these products at a technical meeting, some 300 industrial inquiries were received. Economics, in respect both to cost of the polyol initiator and to minimum requirement for expensive diisocyanate, are favorable, and uniformity of initiator and properties of foams appear acceptable in laboratory tests. However, because foams are made industrially by a continuous process using foam-making machinery, only tests under these conditions can provide a final answer. Arrangements have been made to have such tests conducted by a qualified industrial contractor, who will also engineer a scaled-up process to make polyethers and evaluate quality of the foams produced.

3. Graft copolymers. Research at Stanford Research Institute on kinetics of starch graft polymerization reactions has revealed conditions for ceric ion induced grafting of acrylonitrile that permit as much as 50 percent weight add-on in 10 minutes reaction time. About 92 percent of the added monomer was grafted to starch and only 5 percent to homopolymer. In grafting acrylonitrile to pre-irradiated granular starch, the rate of grafting was found to be limited by the rate of diffusion of monomer into the granules. This rate was greatly increased when starch containing 10 percent moisture was used instead of bone-dry starch.

In addition to this research, arrangements for a second contract project have just been completed with Stanford Research Institute. This project involves process development studies on selected graft copolymers. (A report of an earlier contract project, completed this year, is given in Area No. 2, Part B, Item 4.) In addition, in-house research is being initiated on several basic aspects of the chemistry of graft polymerization, and a PL 480 grant has been made to the Hebrew University, Jerusalem, Israel, for studies on vinyl and epoxide graft copolymers of starch and dextrin.

Research on starch graft copolymers, heretofore a virtually unknown class of starch derivatives, has been highly productive. Results so far obtained indicate that new commercial products will eventuate from this work and that new areas for utilization of starch will be opened up. To take advantage of the utilization opportunities offered by these new products, a considerable concentration of effort is being placed on their study and evaluation.

4. Chemical products from starch and dextrin. Further study of the reaction of starch and its derivatives with vinyl ethers revealed two promising new products. These are a tacky, benzene-soluble gum obtained from starch and isooctyl vinyl ether and a polymeric material obtained from methyl glucoside and ethylene glycol divinyl ether. In addition several new procedures for preparing starch acetals and ketals were explored. Water-soluble products deserving further study were obtained with these procedures by reacting starch with 2,2-dimethoxypropane, crotonaldehyde, and cinnamaldehyde. Planned in-house studies on these reactions have been completed. Further basic research on starch acetal chemistry will be conducted under a grant to Ohio State University Research Foundation.

Hetero-substituted starches, prepared by reacting ten different acidic and neutral groups containing oxygen, nitrogen, sulfur or phosphorus with cationic native and acid-modified starches, failed to display improved deflocculating properties. Indeed a variety of these derivatives, although generally increasing dry tensile strength of paper, behaved as flocculants and had a negative effect on sheet formation. Enzymatic degradation of a low-level cyanoethylated starch gave a promising adhesive for use in high-solids paper coatings.

In contract research at the State University of New York crosslinked, hypochlorite-oxidized corn and wheat starches gave relatively unfavorable results as beater additives in comparison with commercial oxidized starch. These studies have been completed.

At Ohio State University an aminated amylose derivative with a degree of substitution of 1.4 has been synthesized by hydrazinolysis of a di-O-*p*-tolysulfonylamylose followed by reduction of the resultant hydrazino derivative. The modified amylose has been further characterized as its *N*-acetyl derivative. Such a cationic polymer might be expected to possess physical and chemical properties which could offer possibilities for increased utilization of starch. A polymer modified by amination at C-2 might possess the high stability toward hydrolysis exhibited by chitosan; the acetamido analog would be analogous to chitin, a polysaccharide whose high degree of intermolecular hydrogen bonding affords high physical and chemical stability. Studies on the structure of the *N*-acetyl aminated amylose and on other chemical routes to amino and hydrazine derivatives of starch are in progress.

Research is in progress under PL 480 grants on fluorine derivatives of starch at the Hebrew University, Jerusalem, Israel, and on phosphonium and sulfonium derivatives of starch at the National Institute of Technology, Rio de Janiero, Brazil. However, no new or significant results were reported during the year.

At the Institute of Industrial Chemistry, Bologna, Italy, the fatty acid esters of dextrins that have been synthesized were promising oil-soluble surfactants, while some new fatty amine condensation products with corn dextrins were stable surfactants in aqueous media. In studies by the Ahmedabad Textile Industry's Research Association, Ahmedabad, India, starch and karaya gum have been codextrinized and some properties of the product have been measured. These projects are being conducted under PL 480 grants.

5. Evaluation of dialdehyde starch (DAS) and derived products. A low-cost soyflour-blood-DAS adhesive for hot-press bonding of southern pine plywood has been developed. This adhesive has a pH of 9 and a pot life of at least 4 hours. Performance in tests with laboratory-prepared southern pine plywood panels exceeded standard requirements for interior grade plywood.

The highly alkaline protein glues used with Douglas fir are ineffective when used with southern pine. The newly developing southern pine plywood industry is, therefore, without a satisfactory adhesive other than phenolic resins. While the latter would be preferred for exterior grade plywood, they are excessively expensive for interior plywood. The development of the soyflour-blood-DAS adhesive thus is timely and prospects for industrial adoption look very promising. Besides providing an outlet for significant quantities of DAS, the industrial use of the new adhesive would provide a continuing outlet for substantial volumes of soyflour and blood, both of which are, of course, derived from agricultural sources.

Research at Battelle Memorial Institute on evaluation of allyl-DAS (ADAS) has been completed. Considerable potential was indicated for ADAS as a molding resin in filled plastics and as a component of protective coatings based on unsaturated polyester resins.

Performance of ADAS in these applications is sufficiently good to justify further study. Although DAS prices are currently too high for its economical use in ADAS, the price trend is downward. Cost studies at the Northern Division indicate that DAS could be produced at a price low enough to justify its consideration as a raw material for conversion to other products such as ADAS. Development of uses for DAS outside the paper industry, such as ADAS and plywood adhesives, should help accelerate the downward trend in DAS pricing and perhaps assist in interesting new companies in its manufacture.

An interim report from the Quartermaster Field Evaluation Agency indicated that at the end of about 6 months of testing, DAS-tanned leather and regular leather were equivalent in their performance as soles and insoles

in military-type shoes. It is estimated that an additional 18 to 20 months will be needed for completion of evaluative tests of DAS-tanned leather and preparation of a final report by the Quartermaster Research and Engineering Command. Since no further laboratory work at the Northern Division is contemplated in connection with this work, the covering project was discontinued.

In contract research at the University of Minnesota ultracentrifugal studies on borax-dispersed and sodium bisulfite-dispersed DAS have been completed. Excessive degradation of DAS occurred, and the products had low molecular weights (less than 10,000). This property explains many of the results observed with such DAS dispersions.

6. Evaluation of starch derivatives in paper and paper products. Research on new chemical products from starch is supported by evaluation studies to determine the quality and performance of these products in applications in the pulp, paper and paperboard industry. During the reporting period tests were conducted, for example, with starch xanthates and xanthides, hetero-substituted starches, and cyanoethylated starch. Results on the evaluation studies are reported in conjunction with the general discussion of research on the specific starch product.

In addition to these studies, a detailed investigation was made to determine the comparative value of handsheets and machine-made paper for evaluating the performance of cereal xanthides. Statistical analysis of the results showed that replicability of handsheets is poor. Tests on handsheets, therefore, can give evidence only of gross responses of paper properties to changes in variables. Data obtained on machine-made paper show good correlation and provide a good indication of overall properties. A large pilot papermaking machine is being procured and installed at the Northern Division for testing the performance of additives derived from starch.

7. Polymers based on carbohydrates. Studies under a PL 480 grant to the Arthur D. Little Research Institute, Musselburgh, Scotland, have been completed. This work, which has been unusually productive of new information of both basic and practical importance, resulted in the discovery of a variety of novel carbohydrate-derived polymers including both vinyl and nylon (polyamide) types. Because of the potential industrial value of these products and the processes for making them, a number of public service patent applications have been filed covering this work.

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AREA NO. 2: WHEAT
INDUSTRIAL UTILIZATION

Problem. Wheat traditionally commands a higher price than corn. Since the starch content and starch properties of these two cereals are similar, new industrial uses for wheat must rely on advantages to be obtained from other components. Wheat flour is a mixture of starch, protein, gums, fiber, and fat. Because of the simultaneous presence of these basic ingredients, opportunities are promising for development of a wide variety of industrial products from wheat flour that would be expected to have properties and uses different from those of related products derived from refined starch. The problem is to find means for economical modification and reaction of these ingredients with each other and with other chemicals in order to realize the potential of the combinations.

Basic research now being conducted by the Department points to new potential industrial uses for cereal starches and flours that could consume large quantities of grain by 1975. Among potential outlets for wheat flour are sizes for many special grades of paper, cereal pulps that would form an integral part of such papers, and plastic or foamed compositions for hardboard and insulating boards. The opportunity for successful realization of these possibilities is enhanced by recently developed fine-grinding and air-classification milling techniques that permit the composition of flour to be varied over wide ranges. These techniques are now satisfactory for soft wheats, but ways must be found to adapt them to hard wheats which constitute 93 percent of the wheat remaining after current needs have been met.

Wheat flour could achieve its share of potential new markets more rapidly, and discovery of additional new uses under both public and private research programs would be facilitated, if more information were available on the basic physical properties and chemical reactions of flour and its components, on tempering and milling techniques, and on processing methods for economical conversion of flour to desired end products.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing long-range program of research involving analytical, organic and physical chemists, chemical engineers and structural biologists engaged in basic studies of the chemical and physical properties of wheat, flour, flour fractions, and protein components and in applied research leading to new and improved wheat products for industrial use.

The Federal scientific effort for research on industrial utilization of wheat totals 47.0 professional man-years. Of this number 14.6 are devoted to chemical composition and physical properties, 18.6 to industrial chemical products, and 13.8 to processing technology.

Research at Peoria, Illinois, on chemical composition and physical properties (12.2 professional man-years) includes separation, characterization and chemical reactions of the component proteins of wheat gluten. Research contracts (2.4 professional man-years) are in effect at Purdue University, Lafayette, Indiana, for fundamental studies of the alkaline desulfurization of gluten (.8 professional man-year); and IIT Research Institute (formerly called Armour Research Foundation), Chicago, Illinois, for investigation of methods for controlled hydrolysis of gluten (1.6 professional man-years).

Investigations on industrial chemical products conducted at Peoria, Illinois, (14.7 professional man-years) involve preparation and evaluation of new types of water-soluble and water-insoluble flour derivatives for industrial use. Research contracts (3.9 professional man-years) are in effect with Iowa State University, Ames, Iowa, for engineering studies on use of pneumatic fluidization to effect acid modification of flour (.9 professional man-year); and with Battelle Memorial Institute, Columbus, Ohio, for studies on preparation of xanthates from wheat bran and ground whole wheat and their use in making bag and box paper (3.0 professional man-years). During the reporting period contract research was completed at Stanford Research Institute, Menlo Park, California, on graft copolymers from wheat flour and starch and at Iowa State University, Ames, Iowa, on wheat gluten-dialdehyde starch adhesives.

Processing technology research at Peoria, Illinois, (12.6 professional man-years) involves studies on conditioning and milling of wheat, air classification of flours, and reduction of viable microorganisms and radioactive contamination in wheat flour. A research contract (1.2 professional man-years) is in effect with the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on varietal variations in kernel properties and milling and fractionation characteristics of wheat. Contract research has been completed at the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas, on study of the mechanism of enzyme formation during wheat malting and relationship of the information developed to control of enzymes and their action during milling and processing of wheat.

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on processing technology involves a grant to the Cereals Research Station, Research Association of British Flour Millers, St. Albans, England, for investigations on quantitative measurement of properties of wheat that change significantly during conditioning (4 years, 1961-1965). Research under the subheading chemical composition and physical properties, on synthesis of polypeptides compositionally similar to wheat gliadin and corn zein, was completed during the reporting period at the Weizmann Institute of Science, Rehovot, Israel.

PROGRAM OF STATE EXPERIMENT STATIONS

Station research on use of wheat for purposes other than food has been limited. Some work is being devoted to economic feasibility studies related

to use of wheat as a livestock feed when prices are competitive with prices of feed grains. Consideration is also being given to the supply and flow of wheats of different quality.

The total professional man-years devoted to industrial utilization of wheat is .7.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Characterization of wheat gluten proteins. Oxidation in dilute solution of gliadin in which the disulfide bonds had been broken by reduction with thioethanol yielded products that, on the basis of a number of criteria, appeared to be identical to certain components of the original gliadin. Oxidation in more concentrated solution yielded a product very similar, but not identical, to glutenin. Further study of the reoxidation of reduced gamma-gliadin at low urea concentrations indicated that no intermediate products are formed, that only material resembling the original gliadin was obtained, and that the native protein and reoxidized product have similar secondary structure as shown by rotatory dispersion measurements. Immunological tests, conducted with the cooperation of Dr. Douglas Heiner, University of Utah School of Medicine, showed that specific antigenic properties of gliadin, absent in the reduced material, are restored by reoxidation.

Measurement of the molecular weight of gamma-gliadin in the ultracentrifuge by the "equilibrium" method indicated a value of 31,000 in a solvent composed of 6 M guanidine hydrochloride plus 0.1 M acetic acid (pH 3). This value is of the magnitude calculated for the minimum molecular weight from amino acid residues.

Physical chemical studies revealed corresponding changes in viscosity, sedimentation velocity and rotatory dispersion that confirm conformational changes in gluten, glutenin and gliadin at pH 10 previously deduced from hydrogen ion equilibria. In initial studies of the primary structure of the polypeptide chain of gamma-gliadin, enzymatic digestion of the reduced-alkylated protein produced five polypeptides that could be separated by chromatography on Sephadex columns.

The results achieved in this program of research reflect the rapid advances being made in our understanding of the nature of wheat gluten proteins. The information obtained is making an important contribution to the science of protein chemistry. It will also be valuable in future studies on chemical transformations of gluten and flour to new products.

2. Chemical reactions of wheat gluten. The reaction rates of amino groups of various amino acids, peptides and proteins with α,β-unsaturated compounds were shown to be determined by polar and steric constitutive factors according to the Taft-Hammett relationship. The kinetics and mechanism of

alkylation by acrylonitrile of sulfhydryl groups of reduced gluten, peptides, amino acids, and other mercaptans were determined. The second order rate constants for the alkylation were found to be a function of the pH of the media and pK of the individual sulfhydryl groups. The reactive species is probably the $-S^-$ ion which undergoes alkylation much more rapidly than the amino group under comparable conditions.

The reaction of vinyl compounds with proteins to block sulfhydryl groups is used to prevent SH oxidation. The knowledge of factors governing reactivity of sulfhydryls relative to amino and other groups of proteins may facilitate selection of conditions such as pH that will favor specificity for SH groups. The information will also be useful in the grafting of vinyl polymers onto proteins specifically at SH sites.

The reaction of wheat gluten with hydrazine in aqueous solutions resulted in conversion of amide groups of glutamine residues to hydrazide groups. The reaction was accompanied by very little peptide bond cleavage. Partial hydrazinolysis of gluten may yield proteins having markedly different solubility properties. Since aqueous hydrazine is employed the reaction is simple, safe, and economical.

In contract research at IIT Research Institute, vital gluten was 80 percent converted to recoverable soluble polypeptides by maintaining a dispersion of the protein in 0.4N HCl-4N acetic acid at 60° C. for 24 hours. The product had a fairly uniform molecular weight of about 5,000. At Purdue University desulfurization experiments indicated differences in reactivity between laboratory and commercial preparations of vital gluten.

3. Synthetic polypeptides related to wheat gliadin. This research has been completed. A considerable amount of new kinetic data on the polymerization of N-carboxyamino acid anhydrides was obtained. This information will be most useful in studies where model polypeptides are needed to provide better knowledge of the relationship between structure and properties of natural proteins such as gluten and zein. These studies were conducted under a PL 480 grant by the Weizmann Institute of Science, Rehovot, Israel.

B. Industrial Chemical Products

1. Acid-modified flour. Acid-modified flour was prepared in 30-lb. quantities under three sets of conditions: 80° and 110° F. with 4N HCl and 110° F. with gaseous HCl. In machine tests at Forest Products Laboratory, performance of these acid-modified flours was comparable to that of a high-grade commercial surface size except that buildup of viscosity and solids content was greater than previously observed. This could have resulted from moisture absorption by paper with too low an initial moisture content. The carbohydrate-protein ratio in the pastes decreased from 9:1 to about 4:1 at the end of the runs when 90 percent of the pastes had been consumed. However, no gluten balls formed nor did the paper display discoloration or other surface imperfections.

Further trials are needed and planned, but it seems quite possible that completely satisfactory evaluation of these products may not be achieved until suitable papermaking machinery now being procured is installed at the Northern Division. If unequivocably satisfactory performance of these products is eventually established, this demonstration that acid-modified flours made at high temperatures or with anhydrous acid were essentially equivalent to that made at low temperatures, can provide the basis for development of a more economical process.

2. Flour xanthates and xanthides. In addition to starch xanthates and xanthides (see Area No. 1, Part B-1), exploratory studies indicate that the analogous derivatives of wheat flour, bran, ground wheat and related materials produce advantageous improvement in properties when incorporated into pulp and paper products. A mixer-kneader is being used successfully for continuous production of cereal xanthates in the absence of inert diluents. Statistical analysis of 144 runs made with this equipment has revealed the optimum conditions for obtaining xanthates at various degrees of substitution (D.S. 0.34 or less). Chemical efficiencies ranged from 80 to 93 percent depending upon D.S. and time of aging of fresh products.

Preliminary results indicated that aging xanthate products increases the ratio of primary to secondary substitution. However, study of a number of model compounds showed that stability of xanthides of primary and secondary hydroxyl groups does not differ significantly. Since redistribution could affect the performance of xanthides in paper, this phenomenon is being investigated in detail.

When 5 to 10 percent of xanthate (D.S. 0.07-0.13) was incorporated into linerboard, maximum strength properties at high humidity were developed. Other advantageous properties imparted by incorporation of xanthate were high wet strength, improved dry strength, increased stiffness without embrittlement, and water repellency. Further studies on use of *ex situ* crosslinked xanthide indicate that serious stability problems exist for products aged over 24 hours. Because availability of a satisfactory *ex situ* crosslinked product would significantly enlarge the prospective market for cereal xanthides, efforts to develop such materials are continuing.

Use of cereal xanthide to improve crush-resistance of linerboard under conditions of high humidity appears to have good potential for eventual commercial application. Based on available knowledge of the process, cost of using xanthide for this purpose should be less than that of using suitable synthetic resins. A further advantage of xanthide is that it produces desirable stiffness without the embrittlement produced by synthetic resins.

Research on the use of wheat bran and ground whole wheat xanthates in making bag and box papers is being undertaken by Battelle Memorial Institute under a recently negotiated contract.

3. Sulfated wheat flour. Several pounds each of starch and hard red winter wheat flour sulfates, both at two levels of sulfation, were prepared for evaluation on a laboratory fourdinier machine as wet-end additives for paper. Over 300 samples of experimental papers were analyzed chemically to determine retention of the flour and starch sulfates. In general, results on the machine did not compare favorably with previous results on handsheets in that the machine-made papers had poor tensile strength. The poor tensile strength of the machine-made papers is believed to be due to poor formation. This hypothesis will be checked by use of shorter-fibered pulps, which under normal conditions give much better formation than the kraft pulps employed in the present study.

4. New copolymers from wheat starch. Contract research at Stanford Research Institute on graft copolymers has been completed. Final conclusions are that up to 40-weight percent or more of polar monomers such as acrylic acid and acrylamide are readily grafted to starch. Products at all levels of add-on are water soluble. Styrene and acrylic esters also graft readily to starch and at moderate levels give products soluble in organic solvents. With acrylonitrile, products insoluble in all solvents result if add-on exceeds a few percent. Partial hydrolysis of the products, however, yields water-soluble, potentially useful products. With all nonpolar monomers, impractically high levels of add-on are needed to secure moldable products. Partial prehydrolysis of the starch might permit moldability at reasonable levels of add-on (see also Area No. 1, Part B, Item 3).

5. Evaluation of wheat flour products for applications in the pulp and paper industry. Research on chemically modified wheat flour and related products is supported by evaluation studies to determine the quality and performance of these products in applications in the pulp, paper and paperboard industry. During the reporting period tests were conducted, for example, with wheat flour xanthates and xanthides, acid-modified flour, and sulfated flour. Results of the evaluation studies are reported in conjunction with the general discussion of research on the specific wheat flour product.

6. Adhesives from gluten and dialdehyde starch (DAS). Contract research at Iowa State University on DAS-wheat gluten adhesives has been completed. Results showed that adhesives of good tensile strength could be obtained. In a typical procedure the solid product obtained by mixing a dispersion of gluten in acetic acid and one of DAS in sodium bisulfite is redissolved in a slurry of calcium hydroxide in water. This solution, applied to wood, gave an adhesive bond resistant to boiling water and having a tensile strength of 1,068 p.s.i.

In conducting this research, information of value has been developed on solubilities of DAS and wheat gluten, interaction of such dispersions, recovery of the reaction product in solid form, and use of both dispersed and dried reaction products to give adhesives capable of developing practical bond strengths. For practical utility of gluten-DAS adhesives,

further studies will be necessary in order to correlate optimum adhesive properties with practical preparative and use requirements.

7. Fluidization of flour. Contract research was undertaken by Iowa State University to develop information pertinent to use of the fluid bed technique as a means for rapid and effective chemical modification of flour by contact with gaseous or volatilized reagents. Initial results showed that moisture losses during fluidization of flour with dry nitrogen were as high as 50 percent. Addition of 1 to 2 percent of a silicon dioxide anticaking agent reduced the amount of air needed for fluidization.

C. Processing Technology

1. Fine grinding and air classification of wheat flours. Milling and classification studies were extended to four hard red spring varieties of wheat from Montana and North Dakota. Average protein shift was 27 percent in comparison to 49 percent for five popular HRW varieties and 81 percent for three prominent SRW wheats.

Three Ohio SRW wheats were fractionated in sufficient quantity to permit baking evaluation at WU. All showed good response to fractionation. Protein shift was about 86 percent for each. Protein content of the high-protein fraction ranged from 26.9 to 29.1 percent and that of low-protein fractions from 2.4 to 2.7 percent. In contrast, for two durum wheats protein shift averaged 15.6 percent and protein content for high and low protein fractions averaged 26.1 and 10.8 percent, respectively.

Long extraction (85 percent) flours from HRW wheats were suitable for use in the batter process, indicating that the process might be made independent of second-clear supplies. Since users of industrial grade wheat flours in commercial processes have been troubled with raw material shortages, this information could provide an economical solution to the problem.

2. Wheat conditioning. Electron microscopic studies have disclosed a band of material surrounding starch granules in HRW wheat endosperm that stains with osmium tetroxide, suggesting that the band may contain lipids. It has not yet been determined whether this phenomenon is related to the problem of separating starch and protein. This work is being extended to hard red spring wheat and also to soft wheats to obtain some idea of the distribution of lipid and lipoprotein in the protein matrix and possibly elsewhere in the endosperm. A series of extractions with various lipid solvent systems have been made to aid in localizing endosperm lipid and lipoprotein components in electron microscopy.

Immature endosperm of Ponca (HRW) and Selkirk (HRS) was examined at various stages of development. Approximately 10-12 days after pollination, strongly electron opaque bodies began to show in the outermost layer of cells of the endosperm. They increased rapidly in size and number, being quite numerous a few days after their first appearance. Later, these bodies developed into

the globoids of mature aleurone granules. In the essentially mature kernel (30 days after pollination) well-defined vacuoles, mitochondria in various stages of degeneration, and numerous unidentified bodies were still evident in the aleurone layer.

Studies on the effect of conditioning on protein release in wheat were extended to 3° and 30° C. at various moisture levels. Regrinding of straight flours always increased the protein release, however the magnitude of the increase was related to the preceding kernel conditioning treatment; the greatest increase occurred at the lowest conditioning moisture levels. Tempering the flour, rather than the kernel, between 10 to 18 percent moisture, and then regrinding, resulted in about the same degree of protein release as in tempering the kernel. Protein release in flours from immature wheat was somewhat poorer than in mature wheat. In general, little or no protein-free starch was found in starch granules larger than about 13 μ in diameter in flours. Consequently, the problem of protein-release appears to be most acute in relation to the large rather than the small starch granules. Extraction of lipids markedly increased starch-protein binding. However, results of studies of the effect on protein release of incorporating lipids into flours are so far inconclusive.

Research to determine the cause of starch-protein binding in wheat has made significant progress with the confirmation of apparent lipoprotein in the interstitial protein layers and with demonstration that extraction of lipid increases starch-protein binding.

3. Enzymes in malted wheat. Work has been completed at Kansas State University on mode of enzyme formation in wheat germination. Overall results indicate that α-amylase, and tentatively protease, may be synthesized by an oxygen-dependent system stimulated by substances in the developing embryo. The function of these substances can be largely replaced by external treatment with gibberellic acid, thus obviating the need for germination. A new and improved steeping technique (called "roll-steeping") was developed to take advantage of observations that oxidative conditions enhance the effect of gibberellic acid in stimulating formation of α-amylase. The advantage of the technique in malting wheat is that amylase is produced more rapidly in the early stage of germination of the wheat.

4. Reduction of viable microorganisms in flour and flour products. Examination of eight HRW and four HRS wheats and flours therefrom showed only moderate microbial content. By tempering the wheat with chlorine water it was possible to obtain final flours with bacterial counts of not over 1,000 per gram. Fungal counts were reduced similarly. Studies on bacteria and fungi in spoiled and normal refrigerated flour products showed that the predominate bacteria are lactic acid producers of the genus Leuconostoc and that fungi are limited to a few species in the genera Penicillium and Aspergillus.

5. Reduction of radioactive contamination of wheat and milled products. Studies on radioactivity of 1963 wheats showed a level (211 pc/Kg) about three times that for 1962 (83 pc/Kg). Wheat kernels were found to contain less than 10 percent of the total Sr-90 present in the above-ground parts of the plant. Efficient milling gave flour showing only 10 percent of the Sr-90 present in the kernels or 1 percent of that in the above-ground plant. The results indicated that the Sr-90 is located chiefly in the outer bran layers, and that the percentage of the total Sr-90 retained in the flour is lower from wheats that are more highly contaminated by direct fallout. Therefore, it should be possible to mill wheat contaminated by high-density fallout to produce flour suitable for food use. These recently initiated investigations involve cooperation of the Health and Safety Laboratory of the AEC and have as their objective the development of processing methods that would yield nonhazardous wheat products in the event of a nuclear emergency. Present levels of radioactivity in wheat are well within the safe limits established by public health authorities.

6. Quantitative measurement of wheat conditioning variables. Research on this subject, which is being conducted under a PL 480 grant to the Cereals Research Station, Research Association of British Flour Millers, St. Albans, England, was continued during the past year. However, reports describing the work have not yet been received.

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AREA NO. 3: CORN, SORGHUM, AND OTHER FEED GRAINS IMPROVED INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. Abundant quantities of corn, sorghum, and other feed grains are now available beyond those amounts required to satisfy current needs. Both domestic consumption and export potential of these grains could be increased by development of new products for use by industry or of improved foods and feeds. Industrially, increased use of corn and sorghum will be mainly dependent upon increased markets for starch. However, flours derived from these grains are mixtures of starch, protein, and minor amounts of other components. Such mixtures have promise as raw materials for conversion to adhesives, water-soluble coatings, plastic materials, and related products that should have properties and uses different from related products derived from refined starch or wheat flour and that should contribute independently to increasing industrial markets. Isolated protein components of corn and sorghum flours should be suitable raw materials for production of useful resins and films. To achieve these utilization goals, more information is needed on basic physical and chemical properties and reactions of these flours, on the properties of component lipids, waxes, and proteins and their possible interactions with starch, and on the use of fine grinding and air classification and other new milling techniques for obtaining milled products having the most advantageous properties as industrial raw materials.

Because of the growing emphasis on increasing meat production, there is need for processes to obtain improved feed products such as high-protein feeds, mill feeds, feed concentrates, and feeds with high oil content. Such improvement could be achieved through research to obtain better knowledge of the biologically and nutritionally important constituents of corn, sorghum, and oats, to evaluate present, and to develop improved, milling and processing methods, and to ascertain the effects of such methods on the nutritional qualities of the products. In addition, because of the world shortage of protein in human nutrition, this research could enhance the export value of these grains by providing the necessary basis for development of high-protein and other food products that would be acceptable in foreign markets.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-term program involving analytical and organic chemists, chemical engineers and structural biologists engaged in basic studies of the components of corn and sorghum and in application of the new knowledge gained to the development of improved processing technology leading to more effective utilization of these cereal grains.

The Federal scientific effort for research in this area totals 6.1 professional man-years. Of this number 4.2 are devoted to chemical composition and physical properties and 1.9 to processing technology.

Research on chemical composition and physical properties (3.4 professional man-years), conducted at Peoria, Illinois, involves investigations of physiologically active nonprotein nitrogen substances in corn and of carotenoid pigments of corn, corn milling fractions, and yellow endosperm sorghum. A portion of the effort on carotenoid pigments is cooperative with Crops Research Division and is directed to development of corn and sorghum varieties having high carotenoid content. Such varieties are needed for improved food and feed products and to enhance the competitive position of U. S. corn in international trade. A research contract (.8 professional man-year) is in effect with the Indiana University Foundation, Bloomington, Indiana. It provides for studies on the isolation and characterization of phenolic pigments of grain sorghum.

Processing technology research conducted at Peoria, Illinois, (1.9 professional man-years) involves pilot-plant studies of conditions and methods for increasing the yield of oil and grits by dry-milling processes. Effects of processing variations on industrially and biologically important components of corn are determined.

The Department also sponsors research in this area conducted under grants of PL 480 funds to the following foreign institutions: Cereals Research Station, Research Association of British Flour Millers, St. Albans, England, for studies of antioxidants occurring in oats (5 years, 1960-1965); National Institute of Agronomic Research, Paris, France, for basic studies of the physical chemical properties of corn zein (4 years, 1961-1965); and Indian Institute of Science, Bangalore, India, for research on separation of grain sorghum proteins (5 years, 1963-1968). These lines of work are under the subheading chemical composition and physical properties. Research on synthetic peptides compositionally similar to corn zein and wheat gliadin, conducted by the Weizmann Institute of Science, Rehovót, Israel, was completed during the reporting period.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations have a continuing program designed to improve the utilization of corn, sorghum, and other grains in feeds and foods. There is widespread interest in and much effort is devoted to development of varieties of grains with improved nutritive value, pigment content, or other special constituents of value in animal rations. Research on the chemical composition and physical properties of grains is being conducted to support the breeding program on the one hand, and the nutrition program on the other. Development and utilization of corn lines and hybrids with high-protein and high-oil content is an example of the work. The variation in fat and protein content and in amino acid and fatty acid composition is being followed closely. Extraction and characterization of corn proteins

permits selection of corn varieties with improved protein quality and provides information which may lead to new industrial uses.

Related chemical studies are aimed at elucidating the flavor chemistry of corn products. Volatile flavor compounds, generated when corn germ is heated under controlled conditions, are being collected, fractionated and identified.

The work on microbial problems associated with grain storage and utilization involves study of mold deterioration and its effect on wheat and corn. Biological changes are also investigated.

Handling, processing, storage and milling procedures are being researched both from the standpoint of improvement of processing procedures and from the standpoint of effects on ultimate utilization of the products. Processing treatments such as drying, pelleting, enzyme treatment, steaming, dry rolling, and pearling are being studied. Product form, such as whole, cracked, or pelleted, also affects maximum utilization of nutrients and feed efficiency. Product characteristics, such as moisture, protein and fiber content, and pigment retention are also evaluated in terms of value of the grains for feed.

The relative value of sorghum and corn for finishing beef steers is of great significance in the utilization of these grains. Effects of conditioning, drying procedures and storage conditions are important. Studies are in progress to determine the effects of processing grain-type sorghums on their utilization and nutritive value in beef and dairy rations.

Annual crop residues such as corn stalks, sorghum stems, and corn cobs provide a natural reservoir of raw materials. Studies on isolation, characterization and derivatization of hemicelluloses from these sources are in progress in an attempt to modify hemicelluloses in ways to produce new physical properties of potential usefulness.

Study of the economic feasibility and potential market expansion for selected grain crops through new uses and changed utilization patterns is in progress. Both agricultural and nonagricultural uses and particularly uses at processing and manufacturing levels are considered.

Total professional man-years involved in the utilization of cereals and other grains is 4.5.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nonprotein nitrogen substances of corn. Improved isolation and purification procedures yielded apparently homogeneous bound-niacin material from corn gluten. After partial acid hydrolysis of this material, the

niacin is retained on larger fragments containing carbohydrate and amino acids. Phenolic material was also associated with bound niacin. The hull fraction of dry-milled corn contained a high level of bound niacin that can be extracted with 50 percent ethanol. This is an important discovery that should greatly assist further studies on isolation and characterization of bound niacin.

Several mono- and di-phosphate nucleotides were identified in corn extracts. These studies should eventually allow a quantitative study of the morphological distribution of various nucleotides in the mature and immature corn grain. Such study could yield information as to the possible role of the nucleotides as coenzymes in starch biosynthesis and other synthetic process in the developing grain.

2. Corn and sorghum carotenoids. In cooperative studies with Crops Research Division nine corn samples were found that contained 60 p.p.m. or more xanthophyll. One strain contained 68 p.p.m., the highest to date. Data were obtained on carotenoid distribution of various tissues of the corn plant but no correlation was found between that of the kernels and that of other parts of the plant. The progress being made in discovery of corn strains containing high levels of carotenoid pigments is contributing to the ultimate commercial development of such strains, which would have greater value as feeds and would improve the competitive position of U. S. corn in world markets.

Studies on the effects of processing variables on carotenoid content showed that heating 8 hours at 50° C. caused no loss of carotenoids in whole or ground dent and flint corns. However, after 4 hours at 100° C., whole flint corn retained 80 percent of the original xanthophyll level and dent corn 60 percent. Loss in the ground samples was much greater and increased with decreasing particle size. Information of this type should prove valuable both to corn processors as well as to the poultry feed industry.

Research on composition of grain sorghum is being strengthened by a grant which was recently made to the University of Indiana for studies on phenolic pigments of sorghum.

3. Synthetic polypeptides related to corn zein protein. This research, which was conducted under a PL 480 grant by the Weizmann Institute of Science, Rehovot, Israel, has been completed. Valuable basic information pertaining to synthesis of model polypeptides was obtained. This knowledge will be broadly applicable in any future studies relating structure and properties of natural proteins such as zein and gluten.

4. Corn and sorghum proteins. In studies under a PL 480 grant at the National Institute of Agronomic Research, Paris, France, further progress was made in establishing techniques and applying them to the characterization and fractionation of commercial zein. Among the studies reported

were chromatography on DEAE cellulose; light scattering measurements of molecular weight and size; optical rotatory dispersion in aqueous solutions of ethanol, ethylene-diamine, and urea varying in composition; ultraviolet absorption spectrum; dialysis with measurement of passage of zein through the membrane as a function of zein concentration; and column fractionation using a gradient of salt and temperature.

Research on separation of grain sorghum proteins has been initiated under a PL 480 grant at the Indian Institute of Science, Bangalore, India. Samples of three known varieties of grain sorghum were obtained and shown to vary in protein content from 6.5 to 10.6 percent. These samples are being used for preliminary work on extraction and fractionation of proteins.

5. Antioxidants in oats. Work has emphasized chromatographic separation of antioxidant factors isolated from oats and their characterization. A significant development is the recognition that restricting exposure to light eliminates formation of isomeric artifacts. A public service patent application covering synthetic antioxidants similar to those found in oats has been filed. This research is being conducted under a PL 480 grant at the Cereals Research Station, Research Association of British Flour Millers, St. Albans, England.

B. Processing Technology

1. Corn dry milling. Uniform corn degermination tests of four lots of artificially dried corn and of a naturally dried control, demonstrated the adverse effects of drying even under relatively mild conditions. The reduction in yield of premium grits (-3-1/2+6) varied from 20 to 60 percent depending upon severity of drying conditions. Fat content of grits was adversely affected but oil yield increased. Drying 18-percent-moisture corn made it more friable and increased degerminator throughput but with 28-percent corn some "case hardening" offset the friability. The 28-percent-moisture corn also produced less prime goods. With locally grown and dried corn, similar results were obtained except for reduction in both throughput and oil yield. These results indicate that conventional batch and continuous methods for drying corn artificially should be modified because even relatively mild conditions were detrimental for dry milling.

Study of a three-step procedure for tempering low-moisture corn confirmed advantages of a 10- to 20-hour pretemper. Yield of desired -4+6 grits was increased about one-third. Yield of prime goods was also increased slightly, largely because a germ fraction of higher purity was produced. Degerminator throughput was reduced by one-third. Several millers have found pretempering to be an inexpensive method for improving dry milling of corn that has been stored for long periods.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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*Research supported by PL 480 funds.

AREA NO. 4: HIGH-AMYLOSE CORN INDUSTRIAL UTILIZATION

Problem. Varieties of corn have been achieved genetically that contain greatly increased amounts of amylose. Amylose, the linear fraction of starch, possesses film- and fiber-forming properties not available in ordinary starch which contains only about 27 percent of this component. Because the unique properties of amylose open areas of utilization closed to ordinary starch, the potential industrial value of this new crop is very high. Several problems must be solved, however, to realize this potential.

For high-amylose starch to have substantially improved properties as a raw material in comparison with ordinary starch, it should contain at least 80 percent of amylose. Many breeding samples have recently been observed that contain over 80 percent of amylose. However, only varieties containing up to about 75 percent have so far been commercially available. About 5 million pounds of 60 percent high-amylose starch from commercial plantings were utilized by industry in 1962. Although breeding is the task of the geneticist, utilization research is needed to provide information on amylose content, on changes in quantities and properties of the amylose, amylopectin, and other components such as oil and protein, and on milling characteristics of breeding samples in order to insure availability of satisfactory varieties.

To insure utilization of the potentially large volume of high-amylose starch that could eventually become available, more information is needed on the chemical and physical properties of amylose and high-amylose starch and on methods for converting them economically to desired products. Success in this research could lead to an estimated consumption of over several hundred million pounds of high-amylose starch by 1975 in films, fibers, plastics, coatings, and related products to which the linear character of amylose could make contributions.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a long-term, continuing program of research involving analytical, organic and physical chemists, structural biologists, and chemical and mechanical engineers who are engaged in basic and applied research designed to increase knowledge of the properties and reactions of amylose and other components of high-amylose corn and to utilize this knowledge in development of attractive industrial applications for amylose and high-amylose starch.

The Federal scientific effort for research on utilization of high-amylose corn totals 14.7 professional man-years. Of this number 12.3 are devoted to chemical composition and physical properties and 2.4 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (11.3 professional man-years) involves study of amylose content of breeding samples, starch and starch granule composition, structure and properties; and composition and properties of proteins and other components of high-amylose corn. Studies on amylose content of breeding samples assist geneticists in developing varieties of high-amylose corn having increased amylose content. Cooperation with Field Crops Research Branch, Crops Research Division, is maintained in conducting these studies. A research contract (.2 professional man-year) is in effect at Arizona State University, Tempe, Arizona, for basic research on the interaction of "V" amylose with small organic molecules. A grant (.8 professional man-year) has been made to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for basic studies on variations in starch granules of genetically different corn samples. Research on industrial utilization, which is conducted at Peoria, Illinois, is devoted to preliminary studies on new techniques for formation of amylose films having industrially acceptable properties (2.4 professional man-years). Engineering studies on fractionation of high-amylose starch were completed during the reporting period.

PROGRAM OF STATE EXPERIMENT STATIONS

The station phase of the program designed to develop high-amylose corn for industrial uses is largely one of support for the breeding program. The Indiana, Missouri, and Nebraska stations are continuing development of lines high in amylose content. Samples are analyzed, often on an individual plant basis, to determine amylose content. Some additional effort is devoted to development of techniques or processes for separating amylose from other kernel constituents, to study of enzyme systems, and to search for industrial applications.

Total effort devoted to high-amylose corn utilization is about 1.5 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Amylose content of breeding samples. During the reporting period 16,938 samples of high-amylose corn were analyzed. These were supplied under Memoranda of Understanding by the Bear Hybrid Corn Company, by the Missouri Agricultural Experiment Station and by Crops Research Division at the Missouri Station. Of the samples analyzed in the last 6 months of the period, 44.5 percent contained 75 percent or more amylose, thus increasing the number in this range to a total of 6,130 to date. In addition, a total of 517 samples containing over 80 percent of amylose have now been found. The highest value was 84.3 percent. This contrasts with a total last year of 10 and a maximum amylose content of 81.3 percent. (In this report percentages of amylose refer to apparent values determined by iodine

sorption. True amylose content, measured by quantitative fractionation, averages about 80 percent of the apparent value. However, a representative of one of the companies engaged in developing HA corn and HA starch has stated that he is not concerned with the distinction between true and apparent amylose content since, at least in the applications so far of interest, the starch behaves as if the true and apparent values were identical.) These results indicate that the development of high-amylose corn is proceeding to the objective in a satisfactory manner. Moderate quantities of high-amylose starch containing 70 percent of amylose were made available commercially last year. Experimental quantities of seed are expected this year to be available to any processor or industry.

2. Single-kernel analyses. Single-kernel amylose analyses have been made with an estimated precision of ± 0.75 percent (standard deviation) amylose. In a single ear of high-amylose corn, variation in amylose content from the butt to the tip of the ear ranged from 41 to 83 percent. Analyses of different parts of a single kernel of high-amylose corn showed a variation from about 69 to 80 percent amylose in the starch from the tip cap to the crown of the kernel. The amylose content of corn grown from individual kernels which had been subjected to single-kernel analysis failed, however, to show a correlation with the amylose content of the original kernels.

A part of the difficulty may have been due to adverse growing conditions in the greenhouse; further work is needed.

3. Properties of components. Soluble polysaccharides were isolated from waxy, dent, high-amylose and sweet corns. It was observed that these soluble, branched fractions are heterogeneous, i.e., contain approximately equal portions of an amylopectinlike fraction plus a glycogenlike fraction. The sedimentation coefficient of amylopectin is highly dependent upon concentration while that of glycogen is essentially concentration independent. The amylopectin sedimented from the solution if the concentration was low (0.2%) while the glycogen sedimented first if the concentration was high (1.0%). Fractionating the components in waxy and dent corn soluble polysaccharides by centrifugation at high concentrations gave supernatants resembling the corresponding amylopectin. A similar soluble polysaccharide is also present in high-amylose starch. Since these soluble polysaccharides are not produced when starch is autoclaved at 120° C. for 3 hours, they are not artifacts. Instead it is believed that they represent the precursors to starch. It is significant that these precursors are essentially the same for all genetic starches.

In order to examine whether amylopectin exists as an amylopectin-protein aggregate, dispersed aqueous solutions of waxy and dent corn amylopectin were subjected to a proteolytic enzyme. Disruption occurred but stopped when molecular weights around 4 million were obtained for both dent and waxy corn amylopectins. All studies (autoclaving kernels, hydrolysis by enzymes or acid, and proteolytic disruption of aggregates) give extrapolated

molecular weights for dent and waxy corn amylopectin in the range of 4 to 8 million. This molecular weight range is significantly smaller than the initial molecular weights which vary from around 70 million to 2 or 3 billion.

A hydrolytic enzyme requiring phosphate was found to be associated with the starch in waxy maize. This enzyme behaves as an alpha-amylase and not as a phosphorylase. The phosphate initiates both a hydrolysis and a disaggregation of the amylopectin. If the ground kernels are autoclaved prior to dissolution, then hydrolysis is absent but disaggregation occurs. Both hydrolysis and disaggregation are absent if the dispersed aqueous solution of amylopectin is autoclaved, suggesting that the autoclaving of the kernels prevents aggregation of enzyme(s) with the dispersed amylopectin by possibly denaturing the enzyme.

Oxidation with sodium metaperiodate of high-amylose amylopectins from both single cross and double cross hybrids, failed to reveal the presence of α-1,3 glycosidic linkages. Such linkages would prevent reaction with periodate.

At the University of Arizona basic studies on V-type (helical) amylose showed that formation of its hydrate was reversible at 50° C. and above and that, in the hydrate, each water molecule forms more than one bond to the amylose helix.

4. Proteins of high-amylose corn. Research on comparison of proteins of corn genotypes differing in types of starches has been completed. It has been found that variations occur in the protein components of different corn samples but that these variations are not directly related to the genetic factors responsible for differences in amylose content of the starch. Advances were made in techniques for isolation and characterization of the corn proteins as well as in the study of structural relationships. The disulfide linkages in corn protein were the subject of preliminary study, since these linkages presumably are cleaved by the action of sulfur dioxide in the commercial steeping of corn for starch production. The presence of disulfide-linked subunits was shown for both the alcohol-soluble zein and the alcohol-insoluble glutelin fractions. Preliminary studies on the globulin fraction from whole corn showed the presence of at least 17 electrophoretic components and some progress was made in their fractionation.

The results obtained in this investigation provide an excellent foundation of improved techniques and preliminary information for a more detailed study of the chemical structure and properties of the corn proteins.

B. Industrial Utilization

1. Fractionation of high-amylose starch. When steeping time was extended from 24 to 48 hours, recovery of starch by wet-milling single and double

cross hybrids was increased to 90-92 percent. An improved freeze-thaw technique for pretreating high-amylose starch led to recovery of 90-94 percent pure amylose in yields of 90 percent. Cost studies on the procedure for fractionating high-amylose starch by complexing with fatty acids or alcohols were made in final phases of engineering research. Based on a cost for high-amylose starch of 15 cents per pound, these studies showed the process to be uneconomic for 60-percent high-amylose starch and marginal for 70-percent high-amylose starch. Recovered amylopectin was assigned a nominal value of 5 cents per pound in the calculations. Economics of recovery would be improved if amylopectin could command a higher price or if present prices for high-amylose starch decrease as production increases.

2. Amylose films. Treatment of high-amylose starch with dimethyl sulfoxide (DMSO) at 75-85° C. results in a product dispersible in water at 70-95° C. No fractionation or degradation of the starch by this procedure was detected. High-quality cast films were obtained from the aqueous dispersions. Starches containing 59-73 percent of apparent amylose yielded films having tensile strengths of 10,800 to 11,500 p.s.i. Previously, fractionated amylose of 95-percent purity had yielded films with a tensile strength of 10,100 p.s.i. Without the dimethyl sulfoxide treatment, a temperature of at least 120° C. is required for dispersion of high-amylose starch and some degradation of the starch takes place.

In studies of oxygen permeability of 73-percent amylomaize starch films prepared by the DMSO process both unplasticized and 16-percent glycerol-plasticized films showed no measurable permeability at up to 99 percent relative humidity and temperatures of 5° and 25° C. At 100 percent relative humidity permeability of the plasticized film was about one-half that of a comparable uncoated cellulose film. It was demonstrated that water dispersibility of amylose treated by dissolution in DMSO and precipitation with alcohol is due to conversion to V-type amylose. If more economical methods for effecting this conversion can be found, formation of films from aqueous dispersions of the resulting V-type amylose may prove to be a superior method, in contrast to extrusion of amylose pastes, for coating other packaging films or edible products directly or for laminating. Applications such as these are promising approaches to take maximum advantage of amylose film as a barrier to oxygen.

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AREA NO. 5: WHEAT AND CORN
FERMENTATIVE CONVERSION TO NEW INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. By fermentation of cereal grain substrates, new products can be obtained that are not readily available by other means and have promising potential for industrial, agricultural, and food uses. Processes now under development, if brought to successful conclusion, could lead to substantially increased consumption of grain for fermentative conversion to stable viscosity agents for secondary petroleum recovery by flooding of spent oil wells, to new organic acids and enzymes for industrial use, to feed supplements, and to effective biological insecticides and other pesticides that are harmless to man. In addition, there are good possibilities for utilizing fermentation processes to produce new food products that should promote foreign use of U. S. grains.

To accomplish these objectives and to realize the full potential of fermentative techniques for increasing utilization of grain, a broad program of exploratory research is required to find and identify through taxonomic studies species of organisms producing potentially valuable products, to isolate high-yielding strains or develop them by mutation, hybridization or genetic selection, and to develop basic information on culture media, special nutrients, and other factors required for optimum growth of microorganisms and maximum yields of desired products. Continued maintenance and expansion of a collection of pure cultures of well-characterized organisms is necessary for this research. For successful translation of laboratory results into commercially useful processes, more information is needed on new techniques of fermentation, on development of economical methods of growing organisms and handling fermentation processes on a large scale, and on special procedures for efficient isolation and purification of products from fermentative reaction mixtures. Finally, the most appropriate end uses for products must be identified and information obtained on product evaluation and development.

USDA AND COOPERATIVE PROGRAMS

The Department has a long-range continuing program involving analytical and organic chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic research on microorganisms and microbiological reactions and products and in application of both known and newly discovered principles to the development of practical fermentation processes for conversion of cereal grain substrates to useful chemical, biological, feed and food products.

The Federal scientific effort in this area of research totals 63.2 professional man-years. Of this number 19.0 are devoted to basic research on fermentation processes; 21.7 to industrial chemicals; 14.8 to biological pesticides; and 7.7 to feed and food products.

Basic research on fermentation processes conducted at Peoria, Illinois, (18.8 professional man-years) includes study of taxonomy of molds, yeasts and bacteria; factors affecting viability of microorganisms; and microbiological reactions and products. Basic to these investigations and to the Division's entire research program on fermentation is assembly and maintenance in pure culture of a large collection of agriculturally and industrially important microorganisms. Much of the research on microbiological reactions and products is conducted by the Pioneering Laboratory for Microbiological Chemistry. During the reporting period taxonomic studies on Mucorales and Pseudomonas were discontinued, as were screening studies on production of useful chemicals by fleshy fungi. A research contract (.2 professional man-year) with American Type Culture Collection, Washington, D. C., provides for studies on preservation of certain microorganisms for which lyophilization is ineffective.

Research at Peoria, Illinois, on industrial chemicals (19.8 professional man-years) involves fermentative production of microbial gums, organic acids, and other products for use in the chemical industry. This work includes investigation and development of improved or new procedures for conducting industrial fermentations. During the reporting period studies on production of citric acid by fermentation of starch milk from the batter process for wheat gluten were terminated. A research contract (.3 professional man-year) with the University of Arizona, Tucson, Arizona, provides for studies on polymerization of selected fermentation acids and derivatives of fatty acids. Grants (1.6 professional man-years) have been made to Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation (.8 professional man-year) and to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes (.8 professional man-year).

Research at Peoria, Illinois, on biological pesticides (11.6 professional man-years) is devoted to studies on biological insecticides for Japanese beetle and on plant antibiotics. Investigations on biological insecticides for Japanese beetle and on other insect control agents, is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. During the reporting period initial studies on development of methods for large-scale production of spores of the Japanese beetle milky disease organism were completed. The work was re-directed to take advantage of leads developed in the initial studies and of new knowledge stemming from the extensive and specialized program of contract research on this problem. Research contracts (3.2 professional man-years) covering various phases of research on Japanese beetle pathogens are in effect at Michigan State University, East Lansing, Michigan, for study of factors important to large-scale propagation of the pathogens (.5 professional man-year) and for basic research on enzyme activity in sporulation (.7 professional man-year); at Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms (.5 professional man-year); at the University of Minnesota, St. Paul, Minnesota, for fundamental

studies on the transfer of genetic determinants of sporulation from one microorganism to another (.5 professional man-year); at the University of Illinois, Urbana, Illinois, for research on the applicability of a sporulation factor produced by bacteria to Japanese beetle pathogens (.6 professional man-year); and at Baylor University, Houston, Texas, for investigation of morphological changes involved in sporulation (.4 professional man-year).

Research at Peoria, Illinois, on feed and food products (5.5 professional man-years) involves study of production of microbial carotenoids suitable for feed supplements and development of new fermented wheat foods that can help increase export markets for U. S. wheat. Research contracts (2.2 professional man-years) are in effect with the Michigan Agricultural Experiment Station, Michigan State University, East Lansing, Michigan, for evaluation of biological availability of fermentative β-carotene when fed to poultry and swine (.5 professional man-year); and with A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene (1.7 professional man-years).

The Department also sponsors research in the fermentation area conducted by foreign institutions under grants of PL 480 funds. Basic research on fermentation processes involves grants to the National Institute for Agronomic Research, Madrid, Spain, for collection of new species of yeast (5 years, 1960-1965); University of Helsinki, Helsinki, Finland, for basic studies on organic phosphorus compounds of yeast (5 years, 1960-1965); University of Milan, Milan, Italy, for basic studies on the metabolic pathway to 2-keto-gluconic acid in Acetobacter species (4 years, 1960-1964); University of Allahabad, Allahabad, India, for collection of new Mucorales species (5 years, 1961-1966) and studies on survival of lyophilized microorganisms (5 years, 1962-1967); University of Durham, Newcastle-upon-Tyne, England, for investigations of sugar phosphate derivatives in molds (5 years, 1962-1967); and Indian Institute of Science, Bangalore, India, for basic research on enzyme systems involved in Pseudomonas conversion of glucose (5 years, 1962-1967). Research on industrial chemicals involves a grant to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968). Research on feed and food products involves a grant to the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, for research on production of vitamin B_{13} (5 years, 1960-1965); and the National Institute for Agronomic Research, Paris, France, for studies on mutation of yeasts for improved feeds (3 years, 1963-1966). During the reporting period the research on aerobic fermentation was completed by the Superior Institute of Health, Rome, Italy.

PROGRAM OF STATE EXPERIMENT STATIONS

The Montana station is studying conversion of barley into feed yeast protein. Barley carbohydrates are converted enzymatically into fermentable sugars which, in turn, serve as an energy source for the yeast. Current work involves establishing reliable yield and cost data for analysis of the

process. Other research involves fundamental studies of the organisms, the fermentative process and methods for separating desired products from fermentation liquors.

The total research effort devoted to fermentative conversion as a means of utilization is .9 professional man-year.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Research on Fermentation Processes

1. ARS Culture Collection. As of January 1, 1964, the ARS Culture Collection contained 14,071 permanent cultures, an increase of 512 over 1963. During 1963, 2,165 cultures were distributed.

Because of growing interest in toxin-producing strains of fungi, efforts are being made to incorporate more of them into the collection. The permanent collection now has 107 cultures, and the temporary collection over 300 cultures, of molds representing species and genera known to include toxin-producing strains. Research has been initiated on the production of mycotoxins by strains of Aspergillus flavus and related molds.

Several studies under PL 480 projects are in progress. At the University of Allahabad, Allahabad, India, a great many species of Mucorales have been isolated for the first time, and a number of new species and varieties have been found. These include two new species in the genus Mortierella, one in Helicostylum, one in Mucor, one in Dimargaris, one in Piptocephalis and the rediscovery of the genera Thamnocephalis and Linderina. Additional new forms are under study. Also at the University of Allahabad, in research on possible genetic change resulting from lyophilization, assembly of a group of suitable mold cultures has been completed. The strains have been tested for desirable properties, and lyophilization experiments have been initiated. In studies on isolation and characterization of yeasts at the National Institute of Agronomic Research, Madrid, Spain, a variety of desirable but previously unexamined sources have yielded a number of promising cultures. Since the 1960-62 progress report, 425 additional cultures, making a total of 739, have been received for study at the Northern Division.

2. Taxonomic investigations. Research on taxonomy of certain Mucorales (Absidia) has been completed with the discovery of several additional new species. Studies placing special emphasis on the genus Rhizopus have been initiated. Zygosporic strains were found for the first time in heterothallic species of the section Dubiorugorhizopus.

Research on fleshy fungi has also been completed. Structure of the polymer produced by Plectania occidentalis is primarily that of a glucan with β-1,3-linkages and short chains branched via 1,6-linkages. Another, as yet incompletely identified, fungus was found to produce a polymer in 40- to 50-percent yields from glucose, fructose, mannose or sucrose. This polymer

tends to be dark in color and somewhat less viscous than the Plectania polymer. Only glucose seems to be formed by acid hydrolysis.

The new yeast genus Chlamydozyma has provided species ranging from primitive bisexual ones that produce unisexuals readily to more recently evolved bisexual species that yield unisexuals so rarely that the process seems mutational. Good progress is being made in preparation of a monograph on the genus Chlamydozyma. In studies on actinomycete taxonomy, precise determination of color by reflectance measurement was completed for 39 cultures and 79 color standards. Research has been initiated to investigate transfer of genetic materials in microorganisms as a means of increasing their potential as fermentative agents for making useful products from cereal grains.

3. Microbiological processes and products. In the Pioneering Laboratory for Microbiological Chemistry, the agglutination factor on mating type 5 of Hansenula wingei was found to comprise a heterogeneous array of molecules that contain the agglutinative elements. Fractionation gave highly active material having a molecular weight greater than 100 million. It contained about 20 percent protein and 80 percent carbohydrate, and minute amounts per type 21 cell caused agglutination. Studies on energy transfer in cells revealed that a regulatory mechanism exists that determines whether energy is used for ATP synthesis or for reductive processes. This regulatory system is related to variable structural states in the isolated cell particles. The structural states are controlled by the ionic strength of the medium and the redox environment.

In research on feed-back control in microorganisms, a nonspecific acid phosphomonoesterase has been found in Saccharomyces mellis. The formation of this enzyme was repressed if the organism was grown in a medium containing phosphate. The polyphosphate fraction of yeast cells was also affected by phosphate in the medium, but its formation was thereby enhanced. No relationship between phosphomonoesterase and polyphosphate levels appears to exist other than that they are influenced inversely by a common stimulus. Studies on structure of fonsecin, a pigment formed by a mutant of Aspergillus fonsecaeus, have been completed and its constitution has been definitely established. A crystalline compound isolated from A. fischeri was identified as avenaciolide, an antibacterial substance that inhibits germination of seeds and fungal spores.

In studies under a PL 480 grant at the University of Milan, Milan, Italy, most of the enzymic activities which produce both 2-ketogluconic acid and 5-ketogluconic acid from gluconate were found in the particulate fraction of disrupted cells, which also contained the required nicotinamide adenine dinucleotide phosphate (NADP) cofactor. The lesser amount of soluble gluconate dehydrogenases present appeared to be of particulate origin. Because spectrographic analysis indicated that flavins and cytochromes also were present, it is supposed that the dehydrogenase activity that results in the formation of keto acids involves the transfer sequence, gluconate to NADP to flavin to cytochrome to O_2. The 5-ketogenic activity

of cells appeared to be inducible while the 2-ketogenic activity was constitutive. Manipulation of the organisms in fermentations to obtain only 5-ketogluconate in fermentations thus may require genetic modification of the organisms rather than environmental control of enzyme induction.

At the University of Helsinki, Helsinki, Finland, all major phosphate ester components of the yeast Torulopsis utilis detectable by two-dimensional paper chromatography were identified. Changes in the metabolic pattern and orthophosphate assimilation under the influence of physiological conditions, metabolic inhibitors and antibiotics were studied. The major change occurred between 40° and 48° C.; phosphate largely accumulated as α-glycerophosphate, fructose diphosphate and trehalose 6-phosphate. This work is being conducted under a PL 480 grant.

Studies on microbial sugars, conducted under a PL 480 grant at the University of Durham, Newcastle-upon-Tyne, England, have shown that the ribitol teichoic acid from Streptomyces niveus has an irregular structure in which the main unit is a 2- or 4-O-β-glucopyranosyl ribitol phosphate. Also, a new nucleotide, a uridine diphosphate mannose, has been isolated from this organism.

B. Industrial Chemicals

1. Conversion of grains to fermentation media. Corn, wheat, sorghum, corn flour, "Hi-starch No. 2" and batter process starch milk were enzymatically converted, in yields of 90 percent or more, to glucose sirups and substituted for commercial glucose in fermentative production of 2-ketogluconic acid, citric acid, and polysaccharides B-1459 and B-1973. Yields of the acids were about equal to those obtained with commercial glucose. Yields of the polysaccharides were increased even when added nitrogen source was reduced to allow for nitrogen already present in the sirup. An Aspergillus sp. was found to yield 10-12 units/ml. of amyloglucosidase, as compared to less than 1 unit/ml. ordinarily obtained with A. niger (NRRL 337) or 4.5 units/ml. obtained with A. niger under improved conditions developed during the reporting period. Demonstration that equal or superior yields in commercially used fermentative processes can be obtained by substitution of enzymatically converted cereals for commercial glucose indicates that this development should have real industrial value. Discovery of improved conditions for producing higher yields of amyloglucosidase from A. niger and of a new Aspergillus species that yields nearly 50 percent more of the enzyme than A. niger should significantly reduce costs and promote industrial adoption of the process. The higher yields of amyloglucosidase should also lower the cost of production of dextrose which is now largely produced by the enzymatic conversion of starch.

2. Enzymatic modification of wheat flour. Promising tub sizes for paper were prepared by modification of wheat flour through controlled action of indigenous enzymes in the flour or by addition of commercial proteolytic

and amylolytic enzymes. Studies indicated that better paper sizes were obtained when most of the solubilized protein had a high molecular weight. Detailed investigations of HRW, SWW and SRW wheat flours showed that total dispersibility and total carbohydrate solubilized ranged from 50-90 percent while total protein solubilized ranged from 10-100 percent. Although present products compare favorably with a high quality commercial sizing starch, these results suggest that, if molecular weight of solubilized protein can be controlled to avoid excessive degradation, a superior product may eventually be obtained. Considerable work remains to be done, however, to achieve this objective. HRW wheat appeared less suitable than the soft wheats for modification to paper sizes.

3. Studies on continuous fermentation techniques. Further studies on simplifying the nutrient medium for fermentative polysaccharide production have resulted in development of a completely soluble medium. Additional improvement in the medium was achieved by reduction of distillers solubles and substitution of enzymatically produced wheat sirup for commercial glucose. Substantial increase in yield of product was thereby achieved in batch tests.

Microbial polysaccharide B-1459 was successfully produced by continuous fermentation. The operation was conducted for 6 days. Daily yield of product was about 50 percent, while the overall yield including growth phase was about 45 percent. Yields up to 70 percent are obtained by conventional fermentation. In a 12-day, single-stage continuous fermentation, however, the product B-1459 polysaccharide had lower viscosity than that from batch fermentation or from previous continuous fermentations of shorter duration. Continuous fermentation is a complex operation; hence it is not surprising that all pertinent variables are difficult to discover and that variable results may follow. Work so far has demonstrated the basic validity of the concept. Reconciliation of presently observed inconsistencies should, therefore, provide the information needed for development of a satisfactory process.

One commercial supplier has been successfully producing polysaccharide B-1459 in a 20,000-gallon fermentor. Inquiries received indicate growing interest in industrial grade polysaccharides. Under these circumstances, we feel that successful production of B-1459 by continuous fermentation and development of a completely soluble medium are results of the greatest significance. They indicate probable success in the effort to reduce substantially production costs of B-1459 and thus enlarge the potential market for this gum.

The toxicological safety of B-1459 as a potential food additive is being evaluated at the Western Division. Ninety-day tests with male and female beagle dogs at levels of one to two grams per kilogram of body weight per day showed no untoward histopathological findings. Liver and kidney function tests were negative. However, the high level feeding resulted in

a purgative action with some lowering of body weight, serum cholesterol, and hemoglobin and erythrocyte count.

Preliminary tests suggested the ability of this polysaccharide to lower serum cholesterol. Weanling rats were fed high cholesterol diets to which was added either polysaccharide B-1459 or a gum, pectin. Serum cholesterol was lowered in both instances but the polysaccharide had the greatest effect. Liver and fecal cholesterol determinations from these feeding tests are in progress.

In feeding tests with both rats and dogs, polysaccharide B-1459 was found to be an insignificant caloric source. In the case of rats, polysaccharide was quantitatively excreted in the feces within the limits of experimental error. When carbon 14-labeled polysaccharide was fed to individual rats, labeled carbon dioxide appeared in expired air in less than two hours after presenting the diet and accounted for 2 percent of the total polysaccharide ingested during 24 hours. On autopsy, liver, kidney, and muscle were found to be labeled. However, in comparison with various plant gums, polysaccharide B-1459 is very resistant to degradation in the digestive tract.

4. Screening and structure studies on microbial polysaccharides. In screening studies for new microbial polymers, a new broad class of such polymers was discovered. These are characterized by (a) peripherally located xylose removable by acid leaving residual polymer containing mannose and glucuronic acid and (b) conversion by graded acid hydrolysis to the same aldobi- and aldotriuronic acids. Structural studies proved the presence of the D-mannuronic acid moiety in polysaccharide B-1973. Polymers Y-2154, native Y-2448 and deacetylated Y-1401 equaled or exceeded controls in tests for effectiveness as soil-suspending agents. Deacetylated B-1459 and B-1973 appeared to be superior to guar gum as deflocculants for papermaking.

Further studies on the structure of polysaccharide B-1973 showed that it possesses a repeating unit composed of equimolar parts of three sugars: D-glucose, D-galactose, and D-mannuronic acid (potassium salt). Examination of aqueous polysaccharide dispersions revealed that they exhibit birefringence both at rest and when flowing. B-1459 was outstanding in this respect.

5. Fermentation acids. Variable yields (ranging from 26 to 84 percent) of citric acid observed in fermentation of starch milk from the batter process apparently can be attributed to variable content of trace elements in the starch milk and to unexplained sensitivity of spores to storage and growth conditions. Indications are that this fermentation may be too sensitive and delicate for commercial application to these crude starch slurries. Extension of pilot-plant studies of this fermentation, therefore, do not appear justified and the work has been terminated.

Research on fermentative production of itatartaric acid has been initiated under a PL 480 grant to the University of Lodz, Lodz, Poland. Preliminary

reports indicate that cultures of Aspergillus terreus and other strains are being isolated and tested for their ability to synthesize itatartaric acid.

6. Foaming in fermentations. Research on foaming in aerobic fermentations at the Superior Institute of Health, Rome, Italy, has been completed. The final conclusion is that morphology of the organism is more important in determining aeration efficiency than is the geometry of the fermentation vessel. In addition, a method was developed for determining oxygen diffusion rates in the presence of microorganisms. The results of this work, which was conducted under a PL 480 grant, will facilitate research on industrial fermentations.

C. Biological Pesticides

1. Biological insecticides for Japanese beetle. Investigation of factors which effect sporulation of B. popilliae on solid media was continued to determine those that are operative and, also, to search for factors which enhance spore numbers. In addition to the previously demonstrated requirements for sporulation (absence of glucose and source of acetate) are the combined effects of boron, washed agar in the medium and overlaying colonies of the organism on solid media with agar. Although more than 3 weeks are required to obtain spores in the colonies, the number of cells that sporulate is substantially increased. Undoubtedly, by overlaying the colonies with agar, more favorable environment is maintained for the formation of spores, perhaps through restriction of oxygen, provision of moisture, or removal of cell metabolic products. A number of new components have been identified in hemolymph and quantitatively estimated.

Contract research at Michigan State University has progressed in elucidating the nutritional requirements of Japanese beetle pathogens. During this work it was discovered that small amounts of accumulated hydrogen peroxide are lethal to cells. However, use of a biphasic growth system doubled all populations in comparison to the control and increased viability, although the percent of cells remaining alive was relatively low. The results suggest that if a toxic factor, e.g., hydrogen peroxide, is responsible, it operates intracellularly and not through liberation into the medium. It was observed that cells in the log phase of growth have very little capacity to produce hydrogen peroxide whereas those in the stationary phase have considerable capacity for peroxide production. The results also demonstrate that the electron transport system of B. popilliae alters its character as the culture ages. None of several enzymes studied was significantly involved in controlling spore formation. Bacillus popilliae cells were 50 to 100 percent converted to spore-like bodies by 2 to 6 successive passes through a liquid sporulation medium but only 0.6 to 7.2 percent of these spore-bodies were capable of reproducing vegetative cell cultures.

Investigators at the University of Minnesota have constructed a simple gene map for B. subtilis. Regions containing regulator or controller genes, as

contrasted to structural genes, were located. Indications were found that the function of these controller genes may be influenced by environmental factors. Also, evidence was found for existence of genetic regions that control biochemical synthesis of certain compounds required for sporulation. Studies on the lysis observed in B. popilliae led to the conclusion that the dying cells had initiated sporulation but lacked the ability to complete the process. Transfer of sporogeny into asporogenous mutants of B. subtilis and of asporogeny into a sporulating strain has been accomplished.

Contract research in progress at the University of Illinois has demonstrated the presence of a sporulation factor in cells of B. popilliae (strain B-2309S, which sporulates on solid media). This factor was shown to be capable of inducing sporulation in B. cereus.

Studies on stabilizing vegetative cells, recently initiated at Kansas State University, have shown that freezing is entirely unsatisfactory whereas, on the basis of incomplete results, lyophilization shows some promise.

A vast amount of basic information pertinent to the sporulation problem of Japanese beetle pathogens is being accumulated, both through in-house research and through an extensive program of contract research. The contract program, which was designed to bring highly specialized competence to bear upon aspects of the problem such as genetics, enzyme systems, cytology and sporulation factors, has already resulted in important contributions as outlined in the preceding statements of progress. This program has been further strengthened by the recent award of a contract to Baylor University College of Medicine for studies of morphological changes involved in sporulation. With more extensive and precise knowledge of the complicated biological mechanisms involved and of the effects on these of environmental factors, mass production of spores should ultimately be successfully achieved.

2. Plant antibiotics. Screening for plant antibiotics has so far made available for further testing 16 stable, nonpolyenic antifungal materials. Seven have been examined to ascertain their activity spectra. Results ranged from inhibition of 5 fungi by one product to inhibition of 27 by another. This work has reached a point justifying field testing to determine the probable value of the antibiotics discovered.

D. Feed and Food Products

1. Microbial carotenoids. Exploration for a fermentative source of xanthophyll pigments was continued, including analysis of 150 bacterial cell pastes and tests of ability of resting cell cultures to convert β-carotene to xanthophyll. No lutein or zeaxanthin was found in the cell pastes. Five algae, 28 bacteria and 57 molds were tested for conversion of β-carotene but no conversion was observed.

Tests at Michigan State University to evaluate the feeding value of fermentation β-carotene showed that the product is a nutritionally satisfactory source of Vitamin A for both swine and poultry. The conversion of β-carotene to Vitamin A was found to be essentially in accord with reported values. For swine, the conversion of β-carotene to Vitamin A was such that, at the presently estimated selling price for the fermentation product, cost of use in feed would be essentially equal to that of the vitamin itself. For poultry, however, conversion of β-carotene to Vitamin A was much higher, so that cost of using the fermentation product in feeds would be about one-third that of the vitamin. Since poultry feed is the most important potential market, the results of these feeding trials are favorable to more extensive study and evaluation of the product and process by industry.

A contract was recently placed with Arthur D. Little, Inc. for studies on stabilization of fermentative β-carotene products.

2. Fermented wheat foods. In recently initiated studies on fermented wheat foods, tempeh was prepared from HRW and SWW wheats with equivalent results if the wheat was cracked before use. Attempts to use unmilled whole wheat were unsuccessful, but even slight surface modification, as by pearling, gave excellent results. Processing losses were lower for pearled wheat in comparison to cracked wheat. Studies on enzyme systems released by tempeh fermentation organisms have been initiated. One interesting observation is that enzyme activity is much higher in culture filtrates grown on a wheat flour medium as compared to a soybean flour medium. Proteases generated by Rhizopus oligosporus, the tempeh mold organism, have potential industrial interest because they form at acid pH's. These early results on wheat tempeh are very favorable to development of a successful product.

3. Vitamin B_{13}. In research under a PL 480 grant at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, good progress was made on studies of the separation, isolation, and characterization of components of a nutritionally active extract of distillers dried solubles (DDS) obtained by ethanol extraction. Six free phenols were detected. Two of these were isolated, and the empirical formula of one was determined. Two crystalline peptides were isolated from only certain batches of DDS. Therefore, the relationship of these to the nutritional activity appears less certain than that of other components.

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*Research supported by PL 480 funds.

AREA NO. 6: FLAXSEED
INDUSTRIAL UTILIZATION OF LINSEED OIL

Problem. Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Thus, over the years 1950-1960, use of linseed oil in drying oil products decreased from 590 to 351 million pounds because of displacement by synthetic materials capable of better performance. During the same period, consumption of synthetic products in protective coatings increased by 50 percent.

To restore the competitive position of linseed oil, new or expanded markets are urgently needed. Such markets can be achieved by an adequate program of basic and applied research. Recent studies by Department scientists have resulted in commercial manufacture and sale of linseed emulsion paints for exterior use that are competitive with synthetic resin emulsion paints. Use of these new linseed oil paints is expected to expand and assist in maintaining linseed oil in the market for exterior paints, which amounted to 70-75 million gallons in 1962. Another new product from linseed oil to which Department research is contributing is a protective coating for concrete that prevents deterioration from deicers and freezing and thawing in winter. Indications are that use of these two new products has halted the decline in consumption of linseed oil. However, additional research is needed to insure maximum acceptance and consumption of these new coatings and to provide still other new or improved products from linseed oil that can maintain and increase its use in all types of protective coatings, a market amounting to 700 million gallons in 1963.

Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry. To furnish a sound basis for chemical modification, a broad program of basic research on linseed oil is required to furnish new leads and new concepts that will point the way to those products having the best chance for acceptance.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic research and on the chemical reactions of linseed oil and its component fatty acids and in the application of the knowledge gained to the development of new or improved products for the chemical and protective coating industries.

The Federal scientific effort concerned with research on industrial uses for linseed oil total 18.2 professional man-years. Of this number 4.8 is devoted to industrial chemical products and 13.4 to protective coating products.

The current program at Peoria, Illinois, does not include research specifically devoted to chemical composition and physical properties.

Research at Peoria, Illinois, on industrial chemical products (4.8 professional man-years) involves exploratory studies to find new reactions and chemical derivatives and basic and applied research on cyclic fatty acids.

Studies on protective coating products in progress at Peoria, Illinois, (10.4 professional man-years) include investigations on new polymers from linseed oil for use as water-soluble vehicles for coatings and basic and applied research on problems related to development of linseed emulsion paints. Research contracts on protective coating products (3.0 professional man-years) are in effect with Kansas State University, Manhattan, Kansas, for research on the use of linseed oil to protect concrete (.7 professional man-year) and on its use as a single coating for both curing and protection of concrete (.9 professional man-year); with North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota, for investigations of aldehyde oils as components of protective coatings (.4 professional man-year); and with Stanford Research Institute, Menlo Park, California, for studies on properties and reactions of new vinyl copolymers of linseed oil (1.0 professional man-year). Basic investigations on the physical chemistry of linseed emulsion systems were completed during the reporting period by the University of Southern California, Los Angeles, California.

The Department also sponsors research conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves a grant to the Experiment Station for Fats and Oils, Milan, Italy, for studies on minor constituents of linseed oil (5 years, 1960-1965). Research on industrial chemical products is conducted by this institution also under a grant for the investigation of products obtained by thermal polymerization of linseed and other polyunsaturated vegetable oils (4 years, 1960-1964) and by the Regional Research Laboratory, Hyderabad, India, under a grant for exploratory studies on hydroxylation of linseed and safflower oils (5 years, 1963-1968). Research on protective coating products involves a grant to the Paint Research Station, Teddington, England, for fundamental research on organometallic compounds as components of protective coatings (5 years, 1960-1965).

PROGRAM OF STATE EXPERIMENT STATIONS

State stations did not report research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Minor constituents of linseed oil. At the Experiment Station for Fats and Oils, Milan, Italy, research continued on the identification of minor constituents of linseed oils. A triterpenic alcohol originally isolated

from olive oil has been identified as 24-methylene-cycloarthanol. It is found in at least 13 other oils including linseed and soybean oils. The Fitelson-positive compound in linseed oil is a triterpene alcohol of the euphane series but different from any known member of this series. Work has been started on the effect of minor constituents on the spreading and wetting properties of six American and one Italian linseed oils. Interfacial tension measurements on these oils varied from 5.3 to 20.5 dynes/cm. but surface tension values were essentially constant. This work is being conducted under a PL 480 grant.

B. Industrial Chemical Products

1. Cyclic acids. A variety of esters of C_{18} saturated cyclic acid were examined to evaluate their possible potential as synthetic lubricants. Viscosity indexes ranged from 26 to 143 and pour points from -30 to -95° F. Oxidative stability of straight and branched-chain alkyl esters equaled or exceeded that of bis 2-ethylhexyl sebacate. In earlier studies the 2-ethylhexyl ester of saturated cyclic acids showed poor oxidative stability. This result was caused by unrecognized contamination with aromatized cyclic acid ester, since a pure preparation of the latter exhibited much lower oxidative stability than the sebacic acid ester. The saturated cyclic acid used in the present tests contained no aromatic material.

Studies of C_{18} and C_{20} saturated cyclic alcohols in a variety of cosmetic emulsion systems showed that they imparted better feel and esthetic appearance than did cetyl or stearyl alcohols. In antiperspirant formulations containing aluminum salts, the cyclic acids imparted much greater emolliency and reduced tackiness, a persistent problem with such formulations.

Optimum conditions developed in engineering studies on hydrogenation of cyclic acid gave a product containing only 1.1 percent of aromatic product. This level is expected to be satisfactory for most potential uses and to have no significantly adverse effect on oxidative stability of derivatives. Reused hydrogenation catalyst yields less aromatic byproduct than fresh catalyst, a result suggesting the feasibility of a continuous hydrogenation process that would produce little or no aromatic. Purification by a combination of low-temperature crystallization and urea adduct formation appeared to be more practical than either method alone. The limit of effectiveness of liquid-liquid extraction as a means of isolating cyclic acids was apparently reached in current studies with production of a fraction containing 80 percent cyclic acids.

Industrial potential for use of cyclic acids in synthetic lubricants and cyclic alcohols in cosmetics appears to be excellent. Since the cosmetics market can consume premium-priced materials, it could, by absorbing the initial costs of initiating production, assist greatly in establishing saturated cyclic acids as an industrial chemical.

2. Glyceride polymers. At the Experiment Station for Fats and Oils, Milan, Italy, under a PL 480 grant, work has been concerned primarily with structure studies on the dimeric and trimeric acids or esters from heat polymerized oils. Dimer acids have been ozonized and attempts are being made to characterize suitable derivatives of the cleavage products, e.g., aldehyde-acids and their dinitrophenylhydrazones, diacids, etc., by several chromatographic techniques. Information available to date is insufficient to elucidate the structure of the polymeric acids but a number of cleavage product derivatives have been tentatively identified, e.g., butyric acid, dibasic acids from C_4 to C_{11} ($C_9 > C_4 > C_8 > C_{10} > C_{11} > C_5 > C_6 > C_7$) and a non-linear aldehyde containing more than 15 carbon atoms.

3. Hydrogenation of linseed and safflower oils. Research has been initiated under a PL 480 grant to the Regional Research Laboratory, Hyderabad, India. A literature survey was completed, and experimental work has been initiated. However, no significant results have yet been achieved.

C. Protective Coating Products

1. Emulsion paints. Coulter counter data on particle size of 135 emulsion samples were processed by a digital computer. Variables involved included oil viscosity, temperature during preparation, rate and duration of shear and age of emulsion. Results showed that mean particle size decreased with decreasing oil viscosity. Increased mixing time did not alter particle size significantly but the size distribution did become narrower. An additional relationship revealed by the computer is that regardless of viscosity and temperature, oil particle size approaches a limiting value, which may, however, be dependent on type and concentration of the emulsion system and on the rate of shear.

This latter finding represents an important new approach--optimization of the emulsifier system in terms of composition and concentration--to control of the rheological properties of linseed oil emulsions and paints made from them. Studies on pigment interaction showed that aggregation of two pigments will occur at pH values between the isoelectric points of these pigments. This happens because within this range particles of the two pigments will have opposite charges. The results of this fundamental research provide the basis for a practical method for predicting interaction between any two pigments and the conditions under which such interactions would be expected. Commercial interest in linseed emulsion paints continues to increase. Recently a major paint manufacturer of national importance announced test marketing of a linseed emulsion paint. Although a large number of companies have put such paints on the market, these have been the smaller regional paint manufacturers. In a related development, an industrial company has prepared reactive linseed emulsifiers in its pilot plant. These emulsifiers were first prepared at the Northern Division early in its research on linseed emulsion paints.

2. Linseed coatings for concrete. Further study at Kansas State University of the use of linseed oil emulsions in curing fresh concrete showed that ASTM specification C156-55T was met and that at 0.16 lb. of linseed oil per square yard, performance exceeded two commercial products tested.

Preliminary results of tests on use of linseed oil as an antispalling agent to protect air-entrained concrete revealed that uncoated beams made with Florence limestone showed general surface deterioration after 22 freeze-thaw cycles. Beams coated with linseed oil showed very slight surface flaking after 77 cycles. At the end of 22 cycles the uncoated beams were coated with linseed oil, and no further deterioration was observed after 55 additional cycles.

These data on protection of air-entrained concrete with linseed oil coatings are confirming the empirical beliefs regarding the value of this treatment that have already resulted in considerable use of linseed antispalling products. Positive evidence of the value of linseed oil coatings obtained by impartial investigators under scientifically controlled conditions should eliminate skepticism and provide the key to their general acceptance and use on air-entrained concrete. Demonstration that such coatings can stop further deterioration of concrete already damaged by freeze-thaw would justify their use on old highways and other concrete work as well as on new, thus vastly increasing the potential consumption of linseed oil in this application.

3. Water-soluble and other new vehicles based on linseed oil. Research on new polymers and vehicles continued to supply leads to promising products justifying further study and evaluation in protective coatings. In synthesis of various linseed acyl methyl glycosides needed for investigation as water-soluble vehicles, a new blocking group, the methoxy carbonyl group, was discovered. This synthetic method may prove generally useful in organic chemistry. Attempts to prepare the vinyl ether of hydroxy ethyl linseed amide have so far given low yields. Vinyl esters of soybean fatty acids were successfully epoxidized with concentrated hydrogen peroxide and formic acid. The dihydroxyethylamide of unsaturated fatty acids was prepared in yields exceeding 90 percent by sodium-catalyzed aminolysis of linseed oil. Esterification of the product with various dibasic acids yielded resinous polymers showing promise as coating films. Set-to-touch times ranged from 0.75 hour (terephthalic acid ester) to 5 days (dimer acid ester). By reacting an equimolar mixture of tris hydroxymethyl aminomethane and linseed fatty acids, a new oxazolinediol was obtained. This product may be useful as a component of alkyd resins.

Initial results of contract research at North Dakota State University on utilization of aldehyde oils in coatings showed that linseed monoaldehyde oil may give baked and fast air drying films with boron trifluoride catalyst. The crude mixed aldehydes obtained by reductive ozonolysis of soybean methyl esters were reacted with tris hydroxymethyl aminomethane

and then linseed fatty acids to yield drying oils having drying properties inferior to those of similar products prepared with formaldehyde.

4. Organometallic compounds in paints. New vehicles comprising gallate-, tannate-, or pyrogallol-modified vegetable oils, esters and oil-modified alkyd resins have been developed. These vehicles, when applied to unoxidized iron and steel surfaces from solutions in or containing mono-ethers of ethylene glycol, were found to complex with the metal surface and produce durable, anticorrosive coatings. Further studies on adsorptive properties of titanium dioxide pigment revealed that phosphorylated methyl oleate was a superior stabilizing agent for dispersions of rutile in organic paint solvents. Applications for public service patents have been filed on both of these developments as well as on the novel acetoacetate-type vehicles described in last year's report. This research is being conducted under a PL 480 grant by the Paint Research Station, Teddington, England.

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*Research supported by PL 480 funds.

AREA NO. 7: SOYBEANS
FOOD AND INDUSTRIAL USES FOR SOYBEAN OIL

Problem. Soybean oil is now the major edible oil of the United States and the most important source of nutritionally important linoleic acid. However, this oil contains an unstable component (linolenic acid) that limits its use as a liquid oil both domestically and in foreign markets. It is estimated that in 1963 at least 3.8 billion pounds of soybean oil (about 90 percent of total domestic use) was consumed in edible products, of which about two-thirds was consumed in hydrogenated form as margarine and shortening. However, production of soybeans continues to increase rapidly and exceeded 700 million bushels in 1963.

The most promising outlets for oil from this ever-growing production of soybeans appear to be in foreign markets as edible oils and fats and in domestic industrial uses. The potential market for vegetable oils imported by Europe is estimated at over 7 billion pounds by 1966. For soybean oil to capture a growing share of this market, more information is needed to show how to eliminate unstable linolenic acid without loss of nutritive value, to determine the extent to which minor constituents influence flavor and other properties of the oil, and to discover methods for modifying hydrogenated soybean oil to achieve desired functional properties such as melting point and texture. This information would also serve as the basis for improving soybean oil for domestic use both as a liquid oil and in its hydrogenated forms. Some additional consumption in the United States might be anticipated because of extended utility resulting from these improvements, even though consumption of edible fats and oils mainly increases with population growth. To achieve the objective, a broad program of basic and applied research is required to provide more knowledge of the properties of linolenic acid and of minor constituents of soybean oil; of the changes that take place in these and other components during oxidation, hydrogenation, and heating; of the effects of these changes on flavor, nutritive value, stability, and other qualities of the oil; and of the effects of modification of glyceride structure on functional properties of hydrogenated forms of soybean oil.

As an industrial oil, soybean, like linseed oil, is faced with growing competition from synthetic products derived from nonagricultural sources. As an industrial source of linoleic acid, soybean fatty acids must also compete with tall oil fatty acids, a byproduct of paper manufacture. The best opportunity for increasing industrial applications of soybean oil appears, therefore, to be development of products that retain the glyceride structure of the oil. Thus, aldehyde oils, a recent discovery of Department scientists, appear to have a promising future, if current research and development is successful, in the multibillion-pound market for resins, fibers, coatings, plastics, plasticizers, pesticides, and paper and textile chemicals. To achieve the potential industrial value of aldehyde oils and

other soybean glyceride products, more fundamental information is needed on reactions of soybean oil that will preserve the glyceride structure and on the physical and chemical properties of the products. Upon this basis, development of a wide variety of new, industrially useful products should be possible.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic and applied research on edible and industrial uses of soybean oil. A food technologist is also required by the program in connection with organoleptic evaluation of edible oils. Objectives of research on edible soybean oil are to identify undesirable flavor components of the oil, to develop basic information on the chemical changes and mechanisms involved in formation or suppression of these components and to apply the knowledge gained to the development of edible soybean oil having improved oxidative, thermal and organoleptic stability. Objectives of research on industrial utilization are to obtain new information on reactions of soybean oil and its components and to use this information to develop new or improved products for use by the chemical and other industries.

The Federal scientific effort for research on soybean oil totals 33.1 professional man-years. Of this number 9.0 are devoted to chemical composition and physical properties, 12.4 to edible utilization, and 11.7 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (9.0 professional man-years) is concerned with isolation and identification of components affecting flavor, heat, light, and storage stability of soybean oil and its hydrogenated products and with development of new and improved methods of separation and analysis for use in these studies.

Research at Peoria, Illinois, on edible utilization of soybean oil (9.5 professional man-years) involves basic and applied studies on selective hydrogenation and on interesterification followed by selective extraction as means of stabilizing soybean oil by removal of linolenate. Research contracts (2.9 professional man-years) are in effect at IIT Research Institute (formerly called Armour Research Foundation), Chicago, Illinois, for development of heterogeneous selective hydrogenation catalysts (1.4 professional man-years); at Rutgers, The State University, New Brunswick, New Jersey, for basic studies on heterogeneous catalysts (1.0 professional man-year); and at the University of Illinois, Urbana, Illinois, for basic research on homogeneous catalysts (.5 professional man-year).

Research at Peoria, Illinois, on industrial utilization of soybean oil (10.0 professional man-years) involves exploratory studies to find new reactions and products and basic and applied investigations of aldehyde oils and other aldehydic products. A research contract (1.7 professional man-years) is in

effect with Fabric Research Laboratories, Dedham, Massachusetts, for investigations on poly(ester-acetals) and poly(amide-acetals) derived from aldehyde oils.

The Department also sponsors research on soybean oil conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the Institute for Fats and Their Derivatives, Seville, Spain, for research on removal of trace metals from soybean oil with ion-exchange resins (5 years, 1960-1965) and to Gdansk Polytechnic, Gdansk, Poland, for studies on soybean sterols and their effect on stability of the oil (4 years, 1961-1965). Research on edible utilization is conducted under grants to the University of Granada, Granada, Spain, for studies on the effect of processing on frying quality of soybean oil (5 years, 1962-1967) and to Tokyo University, Tokyo, Japan, for research on hydrogenation of soybean oil (3 years, 1962-1965). Research on industrial utilization involves grants to the University of Helsinki, Helsinki, Finland, for studies on separation of pure fatty acids from mixtures such as soybean fatty acids (5 years, 1960-1965); Queen Mary College, University of London, London, England, for basic studies on alkaline cleavage of polyunsaturated fatty acids (4 years, 1960-1964); and the Experiment Station for Fats and Oils, Milan, Italy, for research on oxidation with atmospheric oxygen to obtain new soybean oil derivatives (4 years, 1960-1964).

PROGRAM OF STATE EXPERIMENT STATIONS

Station research on food and industrial utilization of soybean oil involves study of the chemical, physical, and nutritional properties of the oil. Factors involved in heat damage of oils receive special attention. Biological utilization of fatty acid isomers and the manner of absorption, and biological activities of certain trans fatty acids are being investigated. Research directed to isolation, fractionation and chemical identification of the compounds responsible for the reversion flavor of soybean oil continues. The mechanism involved in flavor reversion of soybean oil and analysis of the noncarbonyls compounds in the isolated reversion flavor are being studied.

Other food product research evaluates the effect of use of vegetable oils in bakery products to be held in freezer storage. Oilseed processing conditions and methods of extraction and recovery of oil from oil-bearing seeds are also under investigation.

The total State effort devoted to soybean oil utilization is about 2.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Compositional studies on hydrogenated-winterized soybean oils. Study of the fatty acid composition of liquid and solid (stearine) fractions obtained by acetone fractionation of partially hydrogenated soybean oil showed that the random distribution of linolenic acid in the glycerides prohibited complete removal of this acid from either the stearine or liquid fractions. Linolenic removal depended upon selectivity of hydrogenation. Oleic content of the liquid fractions was relatively constant regardless of the fractionating temperature. Compositional differences were found in linoleic and saturated acids for the liquid and stearine fractions, but trans acids exerted only a minor effect, since they were found in relatively high concentrations in both fractions. Oils hydrogenated to iodine values of 106 and 90 gave crystalline stearine fractions of 2.0 percent and 14 percent at 0° C., 14.8 percent and 34.6 percent at -6° C., and 44.5 percent and 62.3 percent at -16° C., respectively. At these levels of hydrogenation iodine numbers of the solid and liquid fractions differed by 20-25 units.

The stability of soybean oil is markedly improved by the hydrogenation-winterization process because of three important modifications: (1) reduction of total unsaturation, (2) reduction of triunsaturated linolenic acid and (3) removal of residual traces of prooxidant catalyst and catalyst carrier by a microfiltration that occurs when fat particles crystallize out. These suspended trace materials, which are not removable by ordinary filtration, serve as catalysts and centers that promote oxidation.

2. Removal of prooxidant metals. In studies under a PL 480 grant at the Institute for Fats and Oils and Their Derivatives, Seville, Spain, removal of metals from hexane solutions of soybean oil has been greatly enhanced by use of a large-surface, macroreticular ion-exchange resin ("Amberlyst-15"). Iron removed under the best conditions ranged from 2.89 to 3.29 ppm. from oil originally containing 3.34 ppm. Removal of copper and zinc was essentially quantitative. About 0.373 to 0.384 ppm. of manganese was removed from the oil which originally contained 0.516 ppm. A highly significant increase in flavor stability was thereby achieved. The process was most effective with crude soybean oil.

3. Effects of sterols on flavor stability. Experiments at Gdansk Polytechnic, Gdansk, Poland, showed that steroids enhanced autoxidation of soybean oil. However, magnitude of the effect indicated that steroids in the concentrations normally present should not play a significant role in limiting flavor stability. Sterols are chemically modified by the action of bleaching earths, but the products did not catalyze, and might inhibit, autoxidation. During storage of used bleaching earth, the modified sterols were found to regain their original structure.

B. Edible Utilization

1. Selective hydrogenation. In basic studies on sorbic acid as a model compound, exchange of deuterium and hydrogen during homogeneous catalytic reduction with pentacyanocobaltate II was slow and incomplete. Exchange of deuterium with carbon-bonded hydrogen was observed only for the delta-carbon atom. In contrast, complete exchange and equilibration takes place over heterogeneous catalysts. Products of homogeneous hydrogenation of unsaturated fats with cobalt carbonyl resembled those obtained with iron carbonyl. However, during the reaction, there was much less accumulation of conjugated dienes, less selectivity towards linolenate, complete absence of monoene hydrogenation to saturates, less double bond migration and more trans isomerization.

Stable complexes of iron carbonyl and methyl linoleate have been isolated. These complexes hydrogenate rapidly, yielding monoenes and stearate, and also catalyze hydrogenation of methyl linoleate under milder conditions than those required with iron carbonyl catalysis.

In contract studies of heterogeneous catalysis at IIT Research Institute, the NMR method for determining 15,16 double bonds (developed at the Northern Division) has been adopted and found to give satisfactory results. Selectivity ratios of 3, compared to earlier values of 2, have been frequently observed with several metallic catalysts on molecular sieve supports.

At the University of Illinois, homogeneous catalysis of soybean oil esters and methyl linoleate has been achieved with platinum complexes of triphenylphosphine activated with hydrogen chloride and/or stannous chloride. These catalysts are selective in that hydrogenation does not proceed beyond monoene. However, conjugation and cis,trans isomerization take place.

Discovery of stable metal carbonyl-linoleate complexes and of several new homogeneous catalysts for hydrogenation of linoleate opens new possibilities for study that may lead to more effective control of the hydrogenation reaction. Observation of selectivities of 3 in heterogeneous catalysis is encouraging. This selectivity is approaching a value that might yield superior results in the hydrogenation-winterization process.

2. Improving flavor stability. Research on interesterification has demonstrated that soybean oil can be interesterified in the presence of immiscible extraction solvents and fractionation achieved on the basis of unsaturation in the rearranged triglycerides. Thus, potassium t-butoxide was found to be an effective interesterification catalyst for oils in an immiscible solvent mixture of acetonitrile, t-butanol and hexane. After reaction the acetonitrile phase contained more triene than before. Another effective solvent system was phenylacetonitrile-hexane. This solvent pair had a partition coefficient of 6.0, equivalent to an iodine value difference of 19 per theoretical plate. These results justify the basic rationale of the approach and provide a sound basis for future work.

Studies on solvent fractionation of hydrogenated-winterized soybean (HWSB) oil have been essentially completed. Liquid oil yields were 10 to 20 percent greater than those from conventionally fractionated HWSB oils. Yields of 90 percent liquid oil containing below 1 percent linolenic acid were obtained. Overall advantages of the present solvent fractionation technique, in comparison to conventional procedures, include ease of operation, increased yield of low-linolenic fraction, decreased yield of undesired saturated oil, and lower cloud-point of product.

3. Frying quality of soybean oil. In studies under a PL 480 grant at the University of Granada, Granada, Spain, it was found that students could not distinguish between soybean or olive oils used in frying potatoes. Also rats did not distinguish between potatoes fried in different fats. There was no correlation between palatability and fat penetration. Penetration of fats in chips and cubes differed for the same fat; but all fats tested differed in their kinetics of penetration.

4. Partial hydrogenation of soybean oil. Reduction of soybean oil was achieved with copper-chromium, copper-nickel, and copper-chromium-manganese catalysts. The best of these catalysts was reported, on the basis of activity, selectivity and cost, to be copper-nickel (95/5). Linolenic acid amounted to 1 to 2 percent at a level of hydrogenation suitable for preparing a hydrogenated winterized oil. This research is being conducted under a PL 480 grant at the Toyo University, Kawagoe, Saitama-ken, Japan.

C. Industrial Utilization

1. Oxidative cleavage of soybean oil and its fatty acids. Although methanol is an excellent solvent for ozonization, its volatility and solvent characteristics make it unsuitable for industrial use in this application. Laboratory studies of other solvents showed that a 50:50 2-methoxyethanol-acetic acid mixture gives aldehyde yields of 95 percent or more when the ozonolysis products were reduced by catalytic hydrogenation with a palladium-charcoal catalyst.

Engineering studies on preparation of methyl azelaaldehydate (MAZ) were directed towards establishment of conditions for catalytic hydrogenation of ozonized soybean oil methyl esters that would minimize formation of by-product dimethyl azelate (DMA). A MAZ-DMA ratio of 8, the highest so far observed, was obtained with a commercial Lindlar (Pb-poisoned Pd on $CaCO_3$) catalyst. Fresh catalyst and high catalyst concentration favored MAZ formation. Further study showed that, in addition to that formed during hydrogenation, a considerable quantity of DMA was present before catalytic reduction. It might possibly have formed during low-temperature storage of the ozonized soybean esters.

Improvements in color and viscosity of aldehyde oils were achieved by use of a better chemical reduction method and a high vacuum, short contact time, falling-film evaporator for removal of volatile aldehydic byproducts.

To obtain information on effects of metallic contaminants, a series of metals was tested for ability to catalyze polymerization of methyl azelaaldehydate. Copper and iron produced the most polymer; lead, zinc, sodium and aluminum were intermediate; and magnesium, calcium, nickel and chromium gave the least polymer. Cationic resins (H+ form), which can be used to remove metallic contaminants, were found to effect conversion of aldehyde oils to acetals without alcoholysis of glyceryl ester linkages.

2. Aldehyde oil derivatives. In preparation of poly(ester-acetals) from isopropylidene glyceryl azelaaldehydate dimethyl acetal by the newly discovered hydrolysis-polymerization technique, it has been found that the polymer has a carboxylic acid end group formed by hydrolysis of the glyceryl ester. This hydrolysis is competitive with hydrolysis of acetal and ketal groups and terminates the polymerizing chain. Maximum molecular weight obtained so far is 1,540, representing 7 repeating units. When converted to the sodium salt, these polymers show moderate surface activity.

A glass coating showing excellent chemical resistance has been prepared from the poly(ester-acetal) of methyl azelaaldehydate, pentaerythritol and ethylene glycol. In studies on crosslinking of these films on glass, boron trifluoride is the best catalyst so far found that gives satisfactory films at moderate temperatures.

Cooperative studies with the Eastern Division on ester-acetals of azelaaldehydic acid as plasticizers for polyvinyl chloride showed that certain of these compounds have high compatibility and impart excellent low-temperature, mechanical, heat- and light-stabilizing properties to the plasticized polymer.

Study of preparation of 9-aminononanoic acid derivatives was initiated. Butyl 9-aminononanoate was obtained in 70-percent yield by high pressure catalytic hydrogenation of butyl azelaaldehydate in aqueous ammonia-ethanol solution. The excellent results achieved in these initial experiments indicate considerable promise for this route to intermediates for use in polyamides and polyesters.

3. Separation of fatty acids. Research under a PL 480 grant at the University of Helsinki, Helsinki, Finland, indicated that a chemical or physical change in unsaturated fatty acids or their methyl esters that occurs during zone refining may be one of the factors precluding effective separation by this means of polyunsaturated fatty acids derived from soybean or linseed fatty acids. The nature of the change is under study. Linolenic acid of 80 percent purity was obtained from linseed oil by a reasonable crystallization method and indications were that linoleic acid of comparable purity could be obtained from soybean oil.

4. New derivatives. Use of potassium deuterate in place of potassium hydroxide in the Varrentrapp reaction with 10-undecanoic acid has confirmed

the proposed mechanism of discrete steps involving intermolecular reversible 1,3 rearrangements of double bonds on the fatty chain. Other work supports the proposed mechanism for cleavage of the β-hydroxy fatty acid to acetic acid and fatty aldehyde of two less carbon atoms. Autoxidation of 9,10 (10,9)-hydroxyoxo stearic acid in ethanolic potassium ethoxide gave azelaic acid in 77 percent yield. Alkali fusion of 11-ethoxy-undecanoic acid or of omega-hydroxy fatty acid gave good yields of the corresponding dibasic acid with no loss of carbon, and alkali fusion of 9,10-epoxystearic acid gave sebacic and suberic acids as the major dibasic products. These studies are in progress under a PL 480 grant to Queen Mary College, University of London, London, England.

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AREA NO. 8: SOYBEANS
FEED, FOOD AND INDUSTRIAL USES FOR MEAL AND PROTEIN

Problem. Production of soybeans continues to increase rapidly and exceeded 700 million bushels in 1963. For profitable disposition, now and in the future, of the growing supplies of meal from U. S. soybeans, improved feed products and new food and industrial uses are needed. Europe is developing a mixed-feed industry that needs high-protein concentrates. This market could approach that in the U. S. which uses high-protein meal from 450 million bushels of soybeans. For U. S. soybeans to achieve the maximum share of this market, more fundamental information is needed on the proteins and other nutritionally important constituents of soybeans and on the effects of processing on these components. Such information should make possible the production of feeds from soybeans having maximum feeding value that would meet the requirements of foreign markets as well as help maintain or increase the use of soybean feeds in the U. S.

U. S. soybeans could play a dominant role in alleviating the world shortage of dietary protein if more information were available on utilizing soybeans and soybean meal, flour, protein and protein concentrates in food products tailored to meet the nutritional and palatability requirements of foreign markets. That the possibilities are very real for increased utilization of soybeans in foreign food is indicated by recent work of the Department that showed how to use U. S. soybeans for Japanese foods. The result of this work was that a market for selected soybeans for Japan was opened that now exceeds one million bushels per year. If U. S. soybeans are to achieve the maximum share of foreign food markets, basic information on nutritionally important components and effects of processing on these components will be needed. In addition, better knowledge will be required of how to use soybean protein products in foodstuffs that will be acceptable abroad.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-range program involving organic and physical chemists and biochemists engaged in basic research on the characterization of components of soybean meal and protein and application of the knowledge gained to solution of problems encountered in processing and utilization of soybean meal and protein.

The Federal scientific effort on utilization of soybeans and soybean meal and protein totals 14.3 professional man-years. Of this number 7.2 are devoted to chemical composition and physical properties and 7.1 to food products.

Research at Peoria, Illinois, on chemical composition and physical properties (7.2 professional man-years) involves basic studies on isolation and

characterization of components of whey proteins and on heat gelation of alcohol-washed protein. During the reporting period compositional studies on acid-precipitated protein were discontinued.

Research at Peoria, Illinois, on food products (6.7 professional man-years) is devoted to development of information on specially processed soybean products pertinent to their use in high-protein foods for foreign markets. A research contract (.4 professional man-year) at the University of Illinois, Urbana, Illinois, is concerned with investigation of factors possibly present in soybeans that could cause digestive disturbances.

The current program at Peoria, Illinois, does not include research on industrial or feed products.

The Department also sponsors research on utilization of soybeans conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the University of Edinburgh, Edinburgh, Scotland, for investigations on polysaccharides of soybeans (4 years, 1960-1964); to the Weizmann Institute of Science, Rehovot, Israel, for research on complexes between soybean protein and other components of the meal (5 years, 1961-1966); to Kagawa University, Kagawa, Japan, for a chromatographic study of soybean sugars and oligosaccharides (3 years, 1963-1966); and to the University of Tokyo, Tokyo, Japan, for studies on soybean sterols (4 years, 1963-1967).

Research on food products involves grants to the Central Miso Institute, Tokyo, Japan, for studies on miso made from dehulled soybean grits (3 years, 1962-1965); Bar-Ilan University, Ramat Gan, Israel, for studies on miso-type food products for use in Israel (3 years, 1962-1965); Israel Institute of Technology, Haifa, Israel, for evaluation of the quality of isolated soybean protein for use in Israeli foods (4 years, 1962-1966); Japan Tofu Association, Tokyo, Japan, for studies on the use of U. S. soybeans for making tofu (2 years, 1963-1965); Institute of Chemistry, Academia Sinica, Taipei, Taiwan, for investigation on preparing Chinese cheese from soybeans (5 years, 1963-1968); and Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan, for studies on improved strains of Saccharomyces rouxii for making shoyu and miso (5 years, 1963-1968). Research under a contract, financed with PL 480 funds, has been completed by the Japan Shoyu Institute, Tokyo, Japan. The contract provided for comparative evaluations of soy sauces prepared from Japanese and U. S. soybeans.

Research on feed products involves a grant to the Hebrew University, Rehovot, Israel, for basic studies on soybean saponins (5 years, 1961-1966).

PROGRAM OF STATE EXPERIMENT STATIONS

The current station program involves both basic and applied research on soybean protein and meal utilization. Much of the basic research is aimed at characterization of soybean meal and protein. Applications of this

information to utilization is also made through several studies involving feed use of the meal. Basic studies seek to characterize the proteins and identify such biologically active components as proteolytic enzymes and their inhibitors. Other work is being directed to separation and identification of the proteins in soybean whey. Genetic effects as expressed in different varieties are being observed. Peptide structure of the individual purified proteins is investigated. Other researches involve study of the basic mechanisms of the biosynthesis of proteins.

In the area of food use, production of high-protein fermented foods such as tempeh from soybeans is the subject of a pilot study. This involves methods of processing, fractionating or modifying soybeans to produce low-cost, protein-rich foods of value for feeding infants and children.

Extensive economic feasibility studies are in progress. These range from use of meals in livestock feeds to the impact of the common market.

Total research effort devoted to soybean meal utilization is 4.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Acid-precipitated protein. In final phases of work on the major components of acid-precipitated soybean protein improvements were made in purifying the 11S, 7S and other globulins. Evidence was obtained that isolated soybean proteins contain carbohydrates, suggesting the presence of glycoproteins and/or carbohydrate-containing impurities. Because of the potential importance of heat-reversible soybean protein gels, which were discovered during the life of this project, emphasis was shifted at expiration of the project to basic studies on the heat gelation phenomenon.

2. Heat gelation of soybean protein. Initial work under this new project was devoted to further study of alcohol extractables of soybean protein since they appear to contain inhibitory material(s) that prevent formation of heat reversible gels from ordinary acid-precipitated protein. Components of the extractables identified so far include triglyceride, phosphatidyl choline, phosphatidyl ethanol amine, genistein, β-sitosterol-D-glucoside and saponins derived mainly from soyasapogenol B plus small amounts of soyasapogenols A and D.

When isoelectrically precipitated soybean protein was heated in 0.1N HCl, crystalline saponins separated. These were characterized by hydrolysis to soyasapogenols, glucuronic acid, hexoses and arabinose. Crystalline saponins have also been isolated from a number of commercial samples of soybean protein.

A large quantity (50 lbs.) of alcohol-washed protein was prepared for industrial evaluation. It gave a dispersion pH of 7 and a foam of good stability. Demonstration of the presence of saponins in commercial soybean protein suggests a possible basis for development of a method of detection and assay of soybean protein in food products. A successful method for this analysis should lead to increased use of soybean protein in food by eliminating a major problem associated with such use.

3. Whey proteins. Two additional trypsin inhibitors, designated B_1 and B_2, have been isolated from soybean whey. Characterization studies show that they differ from inhibitors A_1 and A_2. The total amount of the four inhibitors represents about 5 percent of the protein content of defatted dehulled meal.

Indications of appreciable varietal differences in trypsin inhibitor activity and in yield of isolatable protein have been obtained. Observation of these varietal differences is a significant discovery that could prove to have commercial importance in processing soybeans to food and feed products.

4. Soybean polysaccharides. Under a PL 480 grant, studies at the University of Edinburgh, Edinburgh, Scotland, on isolation and characterization of polysaccharides in soybean hulls are being completed, and a manuscript on galactomannans has been submitted for publication. Fractionation and characterization of polysaccharides in the cotyledon and germ components of the seed are underway. An extension of the work from 4 to 5 years has been recommended to complete planned studies.

5. Complexes of soybean protein with other meal constituents. During the reporting period work was concerned with the isolation and characterization of carbohydrate complexes with soybean proteins. Preliminary results obtained are very interesting and should yield valuable information about this type of soybean protein complex. This research is being conducted under a PL 480 grant by the Weizmann Institute of Science, Rehovot, Israel.

6. Soybean sterols. Scientists at the University of Tokyo, Tokyo, Japan, under a PL 480 grant, found that sterol glycosides isolated from soybean foots comprised a mixture of campesterol-, stigmasterol- and sitosterol-glycosides. The presence of campesterol-glycoside in soybeans has not been reported previously.

B. Food Products

1. Flavor and nutritive value of soybean food products. An improved taste panel procedure was devised for organoleptic evaluation of various soybean products. The new procedure showed that steamed and puffed soybeans had about the same intensity of flavor and were milder than soybeans treated with ultra-high frequency radio waves. Tasters preferred the steamed beans. Full-fat flour appeared to have the least flavor intensity when steamed

10-20 minutes. Alcohol-extracted flakes had a milder flavor than flakes steamed 20 minutes. Washing defatted meal with 80 percent ethanol or isopropanol was the most effective of many solvent treatments tested. Steam treatment of the washed meal gave a still more bland product.

The component(s) responsible for bitterness in the raw soybean was isolated and tentatively identified as a flavanone(s); a leucoanthocyanin may also be present.

Contract studies at the University of Illinois showed that flatus was produced by defatted soybeans eaten in biscuit form or by whole soybeans canned in tomato sauce. It was further shown, however, that alcohol-washed 70-percent-protein concentrate produces much less flatus per hour than dehulled defatted soybean meal. As a result of this apparent success in substantially removing the factor responsible for flatus production by soybean food products, it should now be feasible to isolate and identify the flatus-producing substance(s) and, therefore, to contribute to understanding of the biological mechanism of flatus production as well as to ultimately develop improved food products.

2. Full-fat soybean flour, UNICEF cooperative program. Accelerated storage tests at 100° F. showed that full-fat flour produced by the extrusion process had good stability for 9 months, corresponding to 1 to 2 years of storage at normal temperature. Vitamin assays showed that thiamine and niacin values were the same as for unprocessed flakes whereas values for commercial products were lower. Organoleptic tests gave preference to the extruded product over a commercial product toasted to the same NSI. Feeding tests with poultry indicated higher biological availability for methionine and cystine in the extruded product in comparison to a commercial full-fat flour.

All the results obtained to date are encouraging for the success of the extrusion process. Clinical tests of extruder-cooked soy flours in 1- and 2-year feeding programs for children up to one year of age were recently initiated in Taiwan and Indonesia under UNICEF sponsorship. If these tests are successful, it is probable that UNICEF will try out the process at overseas locations. However, considerably more study is needed to identify and evaluate the important parameters affecting the process. Such extension of our current work is especially desirable to make possible nutritious and palatable soy flour food products not only for infants but also for older children and adults in protein-deficient developing countries throughout the world.

3. Comparison of U. S. and Japanese soybeans for soy sauce. In studies under a PL 480 contract at the Japan Shoyu Institute, Tokyo, Japan, Japanese and U. S. soybeans were compared at 11 commercial processing plants located in different parts of Japan for quality and yield of shoyu. The results showed that U. S. commercial grade No. 2 soybeans produce shoyu that is equal in quality and slightly higher in yield than Japanese

beans selected for shoyu production. The reason for the higher yield was not determined. The lower cost of U. S. soybeans should give them a definite advantage over domestic Japanese beans. This work has been completed.

4. Studies on miso and tofu. Screening studies at the Central Miso Institute, Tokyo, Japan, indicated certain U. S. soybean varieties are preferable to others for the making of miso. Since proper color is an important characteristic of miso, considerable importance is associated with the cooking of the soybeans prior to fermentation. Harosoy is considered an excellent U. S. variety for making miso. The Japan Tofu Association, Tokyo, Japan, has made laboratory and commercial tests on 12 varieties of soybeans to compare their relative value for making Japanese tofu. The results showed that Hawkeye, Chippewa and CNS-4 gave the best quality and highest yields of tofu. Hawkeye rated somewhat better than the other two. The results for Jackson, Lee, Mandarin and Clark were not uniform enough for satisfactory evaluation and require additional study. Hill, Blackhawk and Comet varieties were found unsuitable for making tofu.

Results obtained at Bar-Ilan University, Ramat Gan, Israel, showed that a product similar in appearance and flavor to miso can be produced from soybean flakes. However, difficulties were encountered with surface growth of Aspergillus oryzae, an unexpected development. Investigations have been made of the use of enzyme preparations along with fermentation. This combination has resulted in a reduction in fermentation time.

At the Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan, research has been primarily devoted to a search for superior strains of Saccharomyces rouxii from various shoyu and miso fermentations. Three strains have been selected that give products with superior flavor. Additional selections have been made from the hundreds of isolates collected over Japan. However, some isolates are not Saccharomyces rouxii and it appears that any superior strains of other osmophilic yeasts should also be tested.

All of these studies were conducted under PL 480 grants.

5. Quality of isolated protein for use in Israeli-type foods. In studies under a PL 480 grant to the Israel Institute of Technology, Haifa, Israel, isolated soybean protein prepared by extracting defatted soybean flakes with 0.03M calcium hydroxide at 55° C. was found to be of high quality in respect to color, taste, and nutritional value. Use of isolated protein at the 40-percent level greatly improved the processing and quality of spray-dried bananas having enhanced flavor. Valuable information has been obtained on the effect of processing variables that affect the functional properties of isolated protein. Such detailed information has not been available heretofore.

6. Chinese cheese. Cultures suitable for preparing Chinese cheese (sufu) from soybeans were isolated from several sources, including strains found at each of the three factories making sufu in Taipei. These strains were evaluated with a laboratory-scale procedure developed for the purpose, and certain strains were sent to the Northern Division. From this work, it is apparent that the factories are actually using Actinomucor elegans although the organism previously had been thought to be a Rhizopus. When tested at the Northern Division, a very excellent product could be made using the laboratory method. This research is being conducted under a PL 480 grant by the Institute of Chemistry, Academia Sinica, Taipei, Taiwan.

C. Feed Products

1. Effects of saponins on nutritional quality of soybean feeds and foods. Scientists at the Hebrew University, Rehovot, Israel, found in studies conducted under a PL 480 grant that the isolated soybean saponins inhibited cholinesterase (i.e., killed fish), chymotrypsin, and proteases (in certain insect larvae). Controlled feeding of these saponins as well as feeding saponin-free soybean meal to mice lead to the conclusion that soybean saponins "are harmless even in concentrations much higher than those" found in the processed feed meal. The enzyme inhibitory effect of these saponins was fully counteracted by mixing them with soybean protein, which appears to complex them.

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*Research supported by PL 480 funds.

AREA NO. 9: REPLACEMENT CROPS
UTILIZATION POTENTIAL - NORTHERN REGION

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical characterization of components of interest to obtain clues to likely end uses; (3) selection of the most promising species followed by additional utilization research to explore uses and demonstrate industrial potential and by additional agronomic research to establish proper cultural practices and to select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of necessity for greater national self-sufficiency, the nation will benefit from availability of optimum, practical crop plants to serve its needs.

To achieve the objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potentials. Work of the Department has already revealed several promising sources of new potentially valuable water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants, and on byproducts from processing, such as oilseed meals.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a long-range continuing program of research involving analytical and organic chemists and chemical engineers engaged in examination of uncultivated plants to find unusual and potentially useful components and in detailed characterization and evaluation studies of selected components that have the greatest industrial potential and that are obtainable from agronomically promising plants. Plants or seeds for this program are obtained by cooperation with Crops Research Division which procures material from domestic and foreign sources by means of collecting

trips or from experimental plantings. Materials from abroad are also made available through Crops Research Division PL 480 projects providing for collecting activities by foreign investigators. All seeds and plants are submitted to a broad chemical-screening program to identify sources of unusual and potentially useful components such as oils, fibers, gums, amino acids and proteins. Components of interest from plants rated by Crops Research Division as having a reasonable agronomic potential for the United States are characterized to obtain clues to areas of utilization of probable interest to industry. On the basis of the results, plants having the highest agronomic potential and containing components of greatest potential industrial value are selected for more intensive utilization research. This utilization research is divided among the four Utilization Research and Development Divisions.

The Federal scientific effort devoted to research on replacement crops at Peoria, Illinois, totals 27.3 professional man-years. Of this number 16.0 are concerned with chemical composition and physical properties; 8.9 with industrial utilization of new oilseeds; and 2.4 with industrial utilization of new gum and fiber plants.

Research at Peoria, Illinois, on chemical composition and physical properties (16.0 professional man-years) involves conduct of the program on screening uncultivated plants for unusual and potentially useful oils, fibers, gums, amino acids and other components; organic chemical characterization of selected fractions and components, especially new oils and fatty acids; and studies on properties of new plant fibers. A research contract providing for screening and analysis of seed oils of Brassica (mustard) and related genera to identify species having greatest erucic acid content and agronomic potential was completed during the period by Montana State College, Bozeman, Montana.

Research at Peoria, Illinois, on industrial utilization of new oilseeds (7.3 professional man-years) involves studies on processing of erucic acid oilseeds to obtain oil and meal and investigations on utilization of erucic acid and its derivatives. A research contract (1.6 professional man-years) is in effect with Southern Research Institute, Birmingham, Alabama, for studies on preparation and evaluation of polyamide resins derived from crambe oil.

Research at Peoria, Illinois, on industrial utilization of new gum and fiber plants (2.4 professional man-years) is concerned with development of methods for recovery of gums from plants; with evaluation of plant gums in industrial applications; and with studies on pulping new fiber plants and evaluation of the pulp in paper, structural boards and related products.

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the Institute of General Chemistry, Warsaw, Poland, for determination of glyceride structure of

erucic acid oils (5 years, 1962-1967); and to the Swedish Seed Association, Svalof, Sweden, to find new erucic acid oilseeds (5 years, 1963-1968).

PROGRAM OF STATE EXPERIMENT STATIONS

Discovery and preservation of valuable plant germ plasm is a continuing objective of the station program in new crops. Much of the research in this area is being done via four regional projects and in cooperation with regional centers. A large portion of the work is cooperative with USDA. Each year many plant introductions are grown and evaluated. Annual and perennial crops possessing potential for industrial or agricultural use are further evaluated for agronomic and chemical qualities. These include crops for paper pulp, drugs, insecticides, polysaccharide gums, and oils rich in acids of unusual structure. Assay of native and introduced tropical plants for products of economic value receives special attention.

Basic aspects of this program involve study of the biochemical and physiological bases for differences in crop plants. Attempts are made to determine if differences in biochemical or physiological processes can be associated with particular factors related to quality. Information concerning carbohydrate transformations is sought through study of carbohydrate formation and enzyme mechanisms.

Horticultural specialty crops are gaining in importance. A number of studies are underway to facilitate rapid development of this industry.

The total scientific effort devoted to replacement crops is 9.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Screening for new industrial oils. Since the last report, screening analyses were performed on 862 seed samples and 289 samples of oil were analyzed. Two new Crepis species contained 27-33 percent of the ene-yne acid first found in C. foetida. Seed oil of Sinapis arvensis, a mustard, contained 54 percent erucic acid--more than most rape and equal to some crambe. Of 29 species of Boraginaceae, 24 contained 6,9,12-triene and 6,9,12,15-tetraene in amounts up to 22 percent and 17 percent, respectively. Seed oil of a new annual species, Euphorbia lagascae, was found to contain 58 percent epoxyoleic (vernolic) acid. Oils from two species, Cardamine impatiens and Myrsinites africana, appear to contain more dihydroxy acids than has been found in other oils so far examined. This research is assuring a flow of new information for agronomists in selecting species for experimental plantings and for use in deciding upon possibilities for future preliminary developmental work.

Contract research at Montana State College has been completed. Six hundred samples were analyzed for erucic acid. These included 22 genera in the mustard family; however, most of the samples were Brassicas. Of the various species studied, B. campestris appeared to have the greatest promise as a producer of erucic acid. However, no species was found equalling crambe in erucic acid content and agronomic potential.

2. Characterization of new seed oils and components. The hydroxy acetylenic acid in Helichrysum oil has been shown to be the new compound 9-hydroxy-trans-10-octadecen-12-ynoic acid. The alkali isomerization product of cis-9-octadecen-12-ynoic acid (from Crepis foetida oil) was shown to be a mixture of trans,cis,trans and trans,cis,cis 8,10,12-octadecatrienoic acids. An acid (31 percent of total) in the seed oil of Calea urticaefolia has been characterized as trans-3, cis-9, cis-12-octadecatrienoic acid. Presence of the allenic grouping (C=C=C) in the dienoid acid of Leonotis nepetaefolia seed oil has been confirmed.

At the Institute of General Chemistry, Warsaw, Poland, under a PL 480 grant, studies are in progress on the triglyceride structure of high erucic acid oils. Oils from three seed samples from different plant sources have each been separated on alumina columns into five fractions, which appear to differ in fatty acid composition. A paper chromatographic procedure and pancreatic lipolysis are being applied to fractions obtained from the alumina columns to elucidate the structure of glycerides present.

3. Characterization of the components of crambe. The product formed by myrosinase hydrolysis of the major crambe thioglucoside has been identified as 5-vinyl-2-oxazolidinethione of opposite absolute configuration to goitrin. In addition to this oxazolidinethione (R-goitrin), 1-cyano-2-hydroxy-3-butene has been isolated and characterized as a second major breakdown product formed in some processed crambe meals. Still other products may be formed depending on conditions. Study of the crystalline protein or polypeptide from crambe seed showed that it comprises a single component having a molecular weight of 5,000 and containing no methionine.

Crambe seed lipase had little activity at room temperature in whole or crushed seed at moisture contents up to 13-15 percent. In samples of whole and cracked seed held at room temperature for 1 year, free fatty acid (as oleic) increased from 0.25-0.30 to 0.8 percent for whole seed and 1.0 percent for cracked seed.

The results of current studies on crambe thioglucosides and enzymes, and on the effects of various treatments of crambe meal emphasize the complexity of the problem involved in removal or deactivation of these physiologically active substances. Conditions have been found for making crambe meal that apparently contains no thioglucoside, oxazolidinethione or enzyme activity and has greatly improved feeding value. However, the nature of the conversion products that remain in the meal is unknown. Furthermore, there

is evidence showing the need for improved methods of analysis. Progress in this difficult field may not be rapid, but results achieved so far justify optimism that the problem will be satisfactorily solved.

4. Screening for new seed mucilages. Examination of 108 new species of seed revealed four species containing over 25 percent of water-soluble mucilage. These species are Cassia javanica, C. hirsuta, Trigonella arabica and T. gladiata. Evaluation tests showed that C. marilandica gum performed as well as guar gum as a wet-end additive in paper handsheets. Endosperm flour for evaluation studies was prepared by dry milling 50 pounds of C. occidentalis seed.

5. Screening for new pulp fiber plants. Statistical studies verified the effect of morphological and compositional differences on the pulping of mono- vs. dicotyledenous plants. Among new accessions in the screening program, a bamboo, Oxytenanthera abyssinica, received a favorable rating, but coastal bermuda grass (Cynodon dactylon), a plant having promising crop potential, had unfavorable pulping properties.

Study has been completed for 4 of 9 varieties of sorghum selected as promising pulping materials. Cellulose values for the four varieties studied were essentially equivalent to that of sugarcane bagasse, and the stalks contained less than one-third as much pith, which is favorable to use of sorghum. Pulps prepared from pith cells and core fibers, each a composite of the four varieties, gave similar handsheets which were, however, inferior in strength to handsheets made from pulp from unfractionated stalks of S. almum. Further mechanical processing and removal of fibro-vascular bundles from the core material provided much lower values for the true pith content of sorghum stalks than had been indicated by initial physical analyses.

As potential pulp crops, sorghums have advantages such as homogeneity in fiber length distribution, fair average fiber length, high yields in the field, light-colored outer bark, and composition resembling that of bagasse, an established raw material. They also have disadvantages such as high solubles content, leafiness, an average of 15 percent pith, and absence of any fibers as long as those of kenaf bast cells. Additional evaluation will be needed before the relative significance of these plus and minus factors can be appraised.

B. Industrial Utilization of New Oilseeds

1. Processing crambe seed. Commercial-scale processing of crambe seed was successfully accomplished when 36 tons of seed were processed by the prepress-solvent extraction procedure in the Sydney, Nebraska plant of Pacific Vegetable Oil Corporation. Engineering information obtained at the Northern Division enabled proper adjustment of the commercial equipment to secure very satisfactory operation. About 13 tons of toasted meal, 10 tons of crude oil and 8 tons of hulls were obtained. About 3.5 tons of

coarse foots, ordinarily recycled in full continuous processing, were also recovered. The oil was refined by PVO without difficulty and sent to the Northern Division for research and distribution for industrial evaluation. Defatted crambe meal prepared in the Northern Division pilot plant with a dry heat enzyme deactivation treatment was fed to lambs at the University of Illinois. Weight gains, digestibility and biological availability of protein were nearly equal to the soybean meal control. Palatability was poor but was improved by addition of molasses. No animals were sacrificed; however, there were no visual indications of toxicity. These results contrast with those of rat-feeding tests at the Western Division which showed that dry heat-processed meal was toxic to rats whereas meals produced by the enzymatic hydrolysis procedure or by methanol extraction appeared to be free of toxic effects in short-term feeding tests. In longer-term tests with rats and in chick feeding tests, somewhat poorer results were obtained. A cooperative agreement has been made with Nebraska AES for study of the feeding value for cattle of the meal obtained in the commercial-scale run at PVO.

2. Studies on utilization of erucic acid. Directed interesterification of crambe oil followed by low-temperature crystallization gave a fraction containing 78 percent erucic acid and representing 65 percent of the erucic acid of the original oil. Twenty diesters were prepared from brassylic acid obtained by oxidative cleavage of erucic acid. Tests of these diesters, conducted at the Eastern Division, indicated that brassylates are excellent low-temperature plasticizers for polyvinyl chloride. Morpholides and piperidides of pure erucic and of mixed crambe acids also were prepared for evaluation as plasticizers. In studies at the Southern Division, these derivatives showed good low-temperature properties and low volatility loss as plasticizers for vinyl chloride-acetate copolymer.

The encouraging results obtained in these utilization studies indicate that good prospects exist for developing new industrial uses for crambe oil and erucic acid.

C. Industrial Utilization of New Fiber Plants

1. Kenaf for pulp and paper. Continued progress is being made in optimizing techniques for preparing, handling, and using kenaf pulps. Comparative refining studies on kenaf, hardwood and softwood sulfate pulps showed that kenaf required significantly less time with either ball-mill or beater treatments. Improvements in preparing and handling kenaf mechanical pulps resulted in a cleaner product, pulps having controlled drainage properties, and avoidance of a byproduct problem. Complete characterization and pulping evaluation of two Nebraska-grown kenaf samples from 7- and 28-inch-row spacings disclosed no differences in pulping potential even though average stalk diameters were quite different in the two samples.

An industrial company is maintaining its interest in kenaf and has initiated plantings this year at seven locations in Alabama. Seed was furnished by Crops Research Division. Other firms have indicated interest in utilization of kenaf, including one concerned with development of harvesting machinery for new crops. If economics prove favorable, one of the more promising areas for commercialization of kenaf would be the South.

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AREA NO. 10: SUGARCANE
PROCESSING AND PRODUCTS - NORTHERN REGION

Problem. Quotas established by the Sugar Act effectively prevent the accumulation of surpluses by limiting production to estimated requirements at stable, low prices for sugar. Prices received by farmers of the United States and Puerto Rico for sugarcane are based upon the recoverable sugar content of the cane; and the rising costs of production and processing make imperative the more efficient recovery of increased amounts of sugar to provide adequate returns for both processors and growers. Currently recovery of 75 percent of the total sugar in the cane is considered satisfactory in Louisiana, and about 83 percent in Puerto Rico and Hawaii. Improved processing methods could increase the recoverable sugar to at least 85 percent in Louisiana and over 90 percent in other areas. The development of more efficient processing methods depends in turn upon the acquisition of adequate data on the quantitative composition of juices extracted from sugarcane, and of materials processed to recover sugar. The chemical industry provides a promising potential for the utilization of additional sugar since more than 15 billion pounds of chemical products are produced annually and sold to every section of American industry. More information is needed on the chemistry and properties of products from sugar to expand their utilization and on the application of these derivatives in the production of plastics, protective coatings, emulsifiers, detergents, and the like.

USDA AND COOPERATIVE PROGRAMS

The major part of the Department's research program on sugarcane processing and products is maintained at the Southern Utilization Research and Development Division, New Orleans, Louisiana. At the Northern Division, Peoria, Illinois, the Department maintains a long-term continuing program involving microbiologists and biochemists engaged in basic and applied research on the fermentative conversion of sugar to industrially useful organic acids.

The Federal program at Peoria, Illinois, totals 2.5 professional man-years, all of which is devoted to new and improved products, specifically, fermentative conversion of sugar to α-ketoglutaric and 2-ketogluconic acids.

In addition, the Department, through the Northern Division, sponsors research in this area under a grant of PL 480 funds to the Institute of Biological Chemistry, University of Rome, Rome, Italy, for studies on the preparation and characterization of dextran derivatives (5 years, 1961-1966). This research is under the subheading, new and improved products.

PROGRAM OF STATE EXPERIMENT STATIONS

Basic and exploratory studies are being carried out at the Puerto Rico station to determine by the use of ion-exchange procedures the possibility of producing sugars that may be utilized in the production of hard candies and similar products without further purification. Experiments will be carried out in pilot-plant scale to evaluate scaling-up the process and to determine operating costs.

Research is also in progress to develop pilot-plant fermentation procedures for use in fermenting as efficiently as possible molasses mashes to produce high quality rums. Other work centers around development of pilot-plant distillation procedures for use in distilling fermented mashes and development of analytical procedures for quality appraisal of rums. A continuous search for new strains of yeast suitable for the fermentation of blackstrap molasses and other materials derived from sugarcane is conducted.

In cooperation with the USDA, several storage and other experiments pertaining to the quality of sorgo juice for sirup and sugar production are conducted annually at the U. S. Sugar Plant Field Station, Meridian, Mississippi. Chemical studies center around total sugars, dextrose, levulose, sucrose and nitrogenous components.

Indiana research seeks to synthesize analogues of important metabolic sugars wherein hetero atoms such as sulfur, selenium or nitrogen replace the normal ring oxygen atom. Sugar analogues and their derivatives will be tested for usefulness as a medicine or as agricultural chemicals.

The research effort on utilization of sugarcane is 3.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. New and Improved Products

1. Production of α-ketoglutaric and 2-ketogluconic acids. Current studies have improved the reproducibility of production of α-ketoglutaric acid with Pseudomonas chlororaphis. Yields, based on glucose, in laboratory-scale equipment are consistently at about 50 percent by weight and occasionally reach 59 percent. Although yields in 20-liter fermentors have not yet reached 50 percent, there appears to be no reason why yields of α-ketoglutaric acid in large-scale equipment should not ultimately equal those obtained in the laboratory. Nevertheless, for the process to be economically attractive, inexpensive means for separating α-ketoglutaric and 2-ketogluconic acids, which are coproducts of the fermentation, will be required. Study of production of 2-ketogluconic acid with Serratia marcescens in 20-liter fermentors was continued. Yields were increased to 90-100 percent by weight in 16 to 24 hours on a medium containing 12 percent glucose. This process gives higher yields in less time than that now used industrially.

Several million pounds of 2-ketogluconic acid are used annually in production of isoascorbic acid, an antioxidant for various food products including meat. A number of industrial companies have requested cultures for making pilot runs or have inquired about the new process, which is the subject of a public service patent.

2. Studies on dextran derivatives. Work has been completed on metal-catalyzed depolymerization of dextran and on the stability of dextran solutions to heat; progress has continued on interaction between dextran derivatives and proteins, dyes, and metal ions. Preparation is being attempted of derivatives having one highly reactive functional group per dextran molecule. This research is being conducted by the Institute of Biological Chemistry, University of Rome, Rome, Italy, under a PL 480 grant.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

New and Improved Products

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*Research supported by PL 480 funds.

AREA NO. 11: FORAGES AND FEED PROCESSING AND PRODUCTS - NORTHERN REGION

Problem. The demand for livestock in the United States will increase 45 percent by 1975. There is an increasing demand for processed forages in European and Asiatic markets. Fresh forage crops are the richest natural source of many nutrients for farm animals. The bulk of forages, however, is preserved so inefficiently by haymaking and ensiling that 10 to 50 percent of the dry weight, and much larger fractions of the most valuable nutrients, are lost before the animals eat them. Dehydration is now the only practical means of preserving the high nutritional value. Poultry and swine producers are aware of the value of dehydrated forage, but restrict their consumption because of high fiber and growth-inhibitor content. The livestock breeder needs forage products tailored to specific animals, and the forage producer must adapt to his needs to sell.

Basic and applied utilization research are necessary to produce: (1) nutritional juice and low-fiber, high-protein feed for non-ruminant animals; (2) fiber products which have been cheaply treated to make them easily digestible for ruminants; (3) growth stimulating supplements for ruminants based on the biologically active fiber digestion factor, and growth-promoting factor in forage. Further, new products should be adaptable to mechanical feeding. Improved handling will encourage farmers to put high value land now producing surplus crops into forages.

USDA AND COOPERATIVE PROGRAMS

The major part of the Department's research program on forages is maintained at the Western Utilization Research and Development Division, Albany, California. At the Northern Division, Peoria, Illinois, the Department has a short-term program involving one organic chemist engaged in research to isolate and identify the toxic component(s) of tall fescue grass responsible for a cattle disease known as "fescue foot." This research is cooperative with the Kentucky State Experiment Station which furnishes toxic and nontoxic fescue grass for chemical study and conducts bioassays of fractions and components isolated from fescue at the Northern Division. Liaison is maintained with the fescue breeding program of the Field Crops Research Branch, ARS, through the Agronomy Department of the University of Kentucky and with the Department's Pharmacology Laboratory at the Western Division.

The Federal program at Peoria, Illinois, totals 1.3 professional man-years, all of which is devoted to chemical composition and physical properties.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct an extensive program of both basic and applied research on forage utilization. Much of the research is interdisciplinary and often involves several departments.

One major segment of the research effort is devoted to determining the chemical composition of forages and evaluation of the relationship between chemical composition of certain forages and their nutritive value for farm animals. Evaluation of the effects of certain agronomic, cultural, processing and handling practices on composition, palatability and nutritive value of forages receives much research attention. Fiber content and utilization of fiber by swine, cattle, sheep and poultry affect the value and use of forages. Methods of isolating and analyzing for fiber are being developed. Investigation of normal and abnormal rumen fermentations of forages is fundamental to maximum utilization.

Careful studies of specific constituents of forages are being undertaken. Determination of certain minor elements found in forages is important both from nutrition and toxicity standpoints. Protein content and quality merit special attention along with determination of amino acid values and unknown growth factors. Leaf organic acids and proteins are investigated in detail in an effort to increase our understanding of their biosynthesis and properties in relation to growth of forage plants.

Due to the economic importance of forages in animal feeds, development of means for evaluation of the nutritive quality of forages has become an important field of study. New and more accurate or rapid chemical procedures are being sought.

Development of forage handling and processing systems to minimize labor costs has led to increased research on forage processing methods. Fermentation characteristics of and animal response to forages which have been wilted, chopped, pelleted, ensiled or dehydrated are being determined. Small-scale ensiling systems are being used to evaluate various silage preservatives. Methods of dehydrating alfalfa are being studied and the economic feasibility of dehydration is being investigated.

The total research effort devoted to forage utilization is about 17.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Fescue toxicity. An unusually large supply--206 bales--of toxic hay was received during the reporting period. Some 2,400 pounds of the hay were extracted with 80 percent ethanol and concentrated for use in

fractionation studies. Samples of an absolute ethanol extract were sent to Kentucky AES for bioassay and to the Western Division and University of Missouri for use in their studies on development of a small animal assay. Indications have been obtained that suggest the possibility that toxin(s) elaborated by microorganisms growing on fescue may be implicated in the disease. This lead is being pursued. In cooperative research at the University of Illinois the structure determination of the major fescue alkaloid was completed by single-crystal X-ray diffraction. It is a position isomer of loline.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

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Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1	Corn, wheat, and other cereal crop utilization investigations--Northern region.			
N1 1-58 (Rev. 2)	Operation and improvement of a culture collection of molds, yeasts, bacteria, and actinomycetes to provide a reservoir of authentic microorganisms for use in making antibiotics, vitamins, chemicals, polymers, assays, and identifications of importance to the national welfare.	Peoria, Ill.	Yes	5-A-1
N1 1-150	Fundamental and exploratory studies of chemical reactions of dextrose and related carbohydrates in nonaqueous solvents basic to development of new and improved industrial products and chemical raw materials from cereal grains.*	Peoria, Ill.	Yes	1-A-1
N1 1-151	Comparative studies on the proteins of corn varieties having starches of widely differing amylose content to provide basic information related to the processing of such new types as high-amylose corn and to the utilization of byproduct protein therefrom.*	Peoria, Ill.	Yes	4-A-4
N1 1-152	Investigation of the characteristics and classification of microorganisms of the genus Absidia and its relatives in the family Mucoraceae, with the exception of Rhizopus, as tools for use in the development of fermentations utilizing cereal crops.*	Peoria, Ill.	Yes	5-A-2
N1 1-153	Exploration of fleshy fungi, algae and plant cells as fermentative agents for making useful products from cereal grains.*	Peoria, Ill.	Yes	5-A-2
N1 1-156(C)	Evaluation of dialdehyde starch as a tanning agent for sole leather.*	Fort Lee, Va.	Yes	1-B-5
N1 1-159	Development of a process for producing in artificial culture media infective spores for use as pesticidal agents against the Japanese beetle.*	Peoria, Ill.	Yes	5-C-1
N1 1-161	Investigation of the taxonomic relationships of bacteria in the Pseudomonas fluorescens species-group which are characterized by ability to oxidize glucose in unique manner, thus to facilitate the production of useful substances, particularly fermentation acids, from cereal grain.*	Peoria, Ill.	No	
N1 1-171(C)	Evaluation of cross-linked hypochlorite-oxidized wheat and corn starches in papermaking as a basis for the development of expanded markets for cereal grain products.*	Syracuse, N. Y.	Yes	1-B-4
N1 1-172(C)	Investigation of the chemical reactions of periodate-oxidized starch (dialdehyde starch) in solutions involved in its potential practical applications as a basis for improving and enlarging its industrial utility.	St. Paul, Minn.	Yes	1-B-5
N1 1-173(C)	Studies on the preparation of graft copolymers from wheat starch and a variety of non-carbohydrate monomers for conversion of starch into new polymers having properties of value for industrial applications.*	Menlo Park, Calif.	Yes	2-B-4
N1 1-175	Investigations on the carotenoid pigments of wet- and dry-milled fractions from corn, including high-amylose types, and of yellow-endosperm sorghum to provide data basic to the most effective utilization of industrial products and fractions from corn and sorghum in feeds.	Peoria, Ill.	Yes	3-A-2

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-176(C)	Polymerization investigations on selected fermentation acids from cereal grains, and on selected fatty acid derivatives from linseed, soybean, and mustard oils, for conversion of these agricultural products to plastics and resins.	Tucson, Ariz.	No	
N1 1-177(C)	Investigations on the interaction of wheat gluten with dialdehyde starches to improve the adhesive properties of gluten.*	Ames, Iowa	Yes	2-B-6
N1 1-178	Investigations on the molecular size and state of aggregation of the amylose and amylopectin components of high-amylose corn starches to provide information basic to industrial utilization.	Peoria, Ill.	Yes	4-A-3
N1 1-179	Basic studies on the chemical structure of the amylose and amylopectin components of high-amylose corn starches to provide information needed for effective industrial utilization of these new starches.	Peoria, Ill.	Yes	4-A-3,B-1
N1 1-180(C)	Investigation of factors required by Bacillus popilliae and Bacillus lentimorbus to produce large and vigorous populations of cells in grain-based media for the mass production of spore dusts to control Japanese beetle infestations.	East Lansing, Mich.	Yes	5-C-1
N1 1-181	Studies of the effects of conditioning treatments of wheat on morphological and histochemical characteristics of milled fractions to provide information basic to the production of industrially useful fractions from wheat.	Peoria, Ill.	Yes	2-C-2
N1 1-182	A comprehensive study of sexual agglutination in yeasts as a basis for developing new yeasts and new processes for the fermentative conversion of cereal grains to new products.	Peoria, Ill.	Yes	5-A-2
N1 1-183	Exploratory studies to convert wheat flour into water-resistant, plasticlike chemical derivatives having properties suitable for industrial use in structural and insulating products and in molding compositions.	Peoria, Ill.	Yes	2-B-2
N1 1-184	Chemical conversion of wheat flour into a variety of hydrophilic polymers having a wide range of solubilities and viscosities in aqueous dispersions to meet specific industrial requirements for sizes, adhesives, and thickeners.	Peoria, Ill.	Yes	2-B-1,3
N1 1-185	Engineering development of a fermentation process for the production of citric acid from the wheat starch slurry of the batter process.*	Peoria, Ill.	Yes	5-B-5
N1 1-186(C)	Studies on the reaction of acetylene with starch and starch-derived products as a basis for development of new products from cereal grains.	Tucson, Ariz.	Yes	1-A-3
N1 1-187	Isolation and characterization of physiologically active nonprotein nitrogenous substances in corn and corn-milling products as a basis for applied processing studies to increase the use of corn.	Peoria, Ill.	Yes	3-A-1
N1 1-188	Investigations on the preparation of acetal and ketal derivatives of cereal starches to obtain starch products having increased water resistance, flexibility, and enhanced solubility in nonaqueous solvents.*	Peoria, Ill.	Yes	1-B-4

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-189(C)	A study of enzyme precursors and the mechanism of enzyme formation during wheat malting to provide basic information needed for the control of enzymes and enzyme action during the milling and processing of wheat.*	Manhattan, Kans.	Yes	2-C-3
N1 1-190(C)	Investigations on methods for the chemical preparation and characterization of amino derivatives of cereal starches by replacement of nonglycosidic hydroxyl groups to obtain new starch products having increased stability to water, dilute acids, and alkali.	Columbus, Ohio	Yes	1-B-4
N1 1-191	Basic taxonomic studies on straight and flexuous streptomycetes of importance to the production of plant antibiotics by fermentation of cereal grains.	Peoria, Ill.	Yes	5-A-2
N1 1-192	Screening microorganisms that may be grown on cereal-based media to produce antibiotics effective against selected fungal diseases in plants, thus providing new fermentation outlets for cereal grains.	Peoria, Ill.	Yes	5-C-2
N1 1-194	Search for microorganisms and a fermentative process to convert cereal grain products to xanthophylls that induce desirable pigmentation of poultry products when added to feed.	Peoria, Ill.	Yes	5-D-1
N1 1-195(C)	Investigations on the alkaline desulfurization of wheat gluten proteins to provide a basis for developing improved modifications of wheat products having utilization potential.	Lafayette, Ind.	Yes	2-A-2
N1 1-196	Chemical investigations on amylomaize selections to guide corn breeders in the development of commercial hybrids containing high-amylose starch for industrial use.	Peoria, Ill.	Yes	4-A-1,2
N1 1-197	Engineering process studies on the acid modification of wheat flour to prepare water-dispersible polymeric products and to make quantities available for product evaluation for use as sizes, adhesives, and thickeners.	Peoria, Ill.	Yes	2-B-1
N1 1-198	Engineering studies on the separation and fractionation of starch from high-amylose corn to prepare a purified corn amylose product for industrial applications and to prepare samples for laboratory studies.*	Peoria, Ill.	Yes	4-B-1,2
N1 1-199(C)	Investigations on control of the chemical hydrolysis of cereal proteins to provide a basis for development of processes to yield polypeptides suitable for industrial uses.	Chicago, Ill.	Yes	2-A-2
N1 1-200	Evaluation of modified cereal flours and starches as sizing agents, coating adhesives, and wet-end additives for paper in large-scale, high-speed continuous runs.	Peoria, Ill.	Yes	2-B-1
N1 1-201(C)	Evaluation of allylated dialdehyde starch as protective and decorative coatings, a molding resin, an adhesive, a modifier for synthetic resins, and as an electrical insulating material for expansion of the industrial use of starch.*	Columbus, Ohio	Yes	1-B-5
N1 1-202	Isolation and characterization of the toxic principle in tall fescue responsible for a cattle disease known as "fescue foot" to provide basic information for increased use of this forage.	Peoria, Ill.	Yes	11-A-1

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-203(C)	Investigations on the control of a selected complex reaction of starch or related carbohydrates through controlled fluid flow dynamics and reaction conditions to provide a basis for process design and improvement leading to increased utilization of cereal grains.	Baltimore, Md.	Yes	1-A-4
N1 1-204	Chemical investigations on the molecular structure of the protein, glutenin, present in wheat gluten as a basis for increased industrial utilization of this raw material.	Peoria, Ill.	Yes	2-A-1
N1 1-205(C)	Investigations on reactions of difunctional mercaptans with dextrose, starch, or related carbohydrates to form polymers having potential industrial value.	Tucson, Ariz.	Yes	1-A-3
N1 1-206(C)	Evaluation of beta-carotene product from fermentation of grain-based media with Blakeslea trispora as vitamin A source for poultry and swine.	East Lansing, Mich.	Yes	5-D-1
N1 1-207	Investigations on the enzymatic modification of wheat flour and flour fractions by combined amylases and proteases to provide pastes of suitable viscosities for use as surface sizes and coating agents for paper.	Peoria, Ill.	Yes	5-B-2
N1 1-208	Investigations on the conversion of cereal grains to economical and efficient soluble fermentation substrates through the action of microbial enzymes, as a basis for increasing the use of these grains by the fermentation industry.	Peoria, Ill.	Yes	5-B-1
N1 1-209	Investigations on the applicability and evaluation of chemically modified cereal grain flours and fractions as ingredients, agents, and adhesives in pulp and paperboard products as a basis for increasing industrial use of cereal grains.	Peoria, Ill.	Yes	1-B-6; 2-B-1,2,3,5
N1 1-210	Investigations on the preparation of water-dispersible hetero-derivatives of starch to obtain products having a wide range of properties for the production of adhesives, sizings, and other additives for applications in paper and related industries.	Peoria, Ill.	Yes	1-B-4
N1 1-211	Chemical reaction studies on wheat gluten and its component proteins seeking methods of modification to give properties better suited for industrial uses.	Peoria, Ill.	Yes	2-A-1,2
N1 1-212	Investigations on the production of low-density plastic foams from starch-derived glucosides and related starch derivatives as a basis for increasing the industrial utilization of cereal starches.	Peoria, Ill.	Yes	1-B-2
N1 1-213	Pilot-plant investigations on wheat dry-milling and fractionation methods for producing a wide variety of products for use in foods, feeds, and industrial products.	Peoria, Ill.	Yes	2-C-1
N1 1-214(C)	Engineering studies on the application of pneumatic fluidization to the reactions of wheat flour with hydrogen chloride as a basis for producing sizing agents for paper.	Ames, Iowa	Yes	2-B-7
N1 1-215	Investigations of the reaction of dialdehyde starch with casein, soybean protein, soy flour and dried animal blood for the production of improved wood adhesives.	Peoria, Ill.	Yes	1-B-5

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-216	Pilot-plant investigations of dry-milling operations to obtain increased yields of prime goods and oil from old and artificially dried corn and to develop a prototype corn degerminator having improved corn degerminating characteristics for production of higher quality dry-milled products.	Peoria, Ill.	Yes	3-B-1
N1 1-217	Investigation of methods for producing microbial polysaccharides from cereal grains by continuous fermentation to reduce production costs allowing increased utilization of these potentially useful gums.	Peoria, Ill.	Yes	5-B-3
N1 1-218(C)	Stabilization of vegetative cells of Bacillus popilliae grown on cereal-based media for use as an infecting agent against the Japanese beetle.	Manhattan, Kans.	Yes	5-C-1
N1 1-219(C)	Study of role of enzymes and enzyme activity in the formation of spores of Bacillus popilliae and Bacillus lentimorbus as a basis for the mass production of biological insecticides by fermenting cereal grain.	East Lansing, Mich.	Yes	5-C-1
N1 1-220(C)	The transfer of genetic determinants of sporulation from one microorganism to another, as a basis for applied studies on the fermentative production of spore dusts for the control of Japanese beetle infestations.	Minneapolis, Minn.	Yes	5-C-1
N1 1-221(C)	Study of the sporulation factor produced by bacilli and its possible use in Bacillus popilliae and Bacillus lentimorbus to develop a fermentation process for the production of spore dust to control Japanese beetle infestations.	Urbana, Ill.	Yes	5-C-1
N1 1-222(C)	Studies on the mechanism and kinetics of radiation and ceric ion induced grafting of cereal starches with vinyl-type monomers previously shown in exploratory studies to graft readily and efficiently with promise for new industrial outlets for starch.	Menlo Park, Calif.	Yes	1-B-3
N1 1-223(C)	Development studies on the semi-pilot-plant scale production of cereal grain xanthides and their use and evaluation in making corrugating board and linerboard for corrugated boxes.	Columbus, Ohio	Yes	1-B-1
N1 1-224	Development of methods and processes to reduce viable microorganisms in wheat flour as it is produced in the mill.	Peoria, Ill.	Yes	2-C-4
N1 1-225	Investigations on the development of new fermented wheat foods through the use of Oriental-type food molds as a basis for increasing export markets for U. S. wheats.	Peoria, Ill.	Yes	5-D-2
N1 1-226	Investigations on formation and properties of amino and peptide derivatives of starch to provide a basis for the development of industrially useful products from cereal grains.	Peoria, Ill.	Yes	1-A-2
N1 1-227	Investigations on the conversion of cereal xanthates to xanthides in physical forms suitable for use in papermaking.	Peoria, Ill.	Yes	1-B-1
N1 1-228(C)	Investigations on the interaction of "V" amylose with small molecules to provide basic information on the helical structure of amylose from high-amylose corn starch.	Tempe, Ariz.	Yes	4-A-3
N1 1-229	Investigations on processing methods for wheat to minimize radioactive contamination in milling products.**	Peoria, Ill.	Yes	2-C-5

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-230(C)	Investigations on the synthesis of terminal C4-modified maltooligosaccharides for use in studying enzyme modifications of cereal starches.**	Carbondale, Ill.	No	
N1 1-231(C)	Stabilization of beta-carotene in dried mycelium and in extracted form as a contribution to commercialization of beta-carotene produced by fermentation of cereal grain.**	Cambridge, Mass.	Yes	5-D-1
N1 1-232(C)	Investigations on the vinylation of methyl glucoside by reaction with acetylene and on the properties and reactions of the products as a basis for development of new industrial outlets for cereal grains.**	Tucson, Ariz.	No	
N1 1-233(Gr)	Studies on the types and variations of starch granules within the endosperm of genetically different high-amylose corns to provide fundamental information important to the processing and utilization of high-amylose corn.**	Lincoln, Nebr.	No	
N1 1-234(Gr)	Investigations of two-phase submerged fermentation processes as means for increasing yields and/or concentrations of products obtained by fermentation of cereal grains.**	Ithaca, N. Y.	No	
N1 1-235(C)	Investigation of the morphological changes involved in the transition of Bacillus popilliae from vegetative cells to spores for controlling Japanese beetle infestations.**	Houston, Tex.	Yes	5-C-1
N1 1-236(C)	Investigation on the isolation and characterization of phenolic pigments of grain sorghum to provide basic information related to the discoloration or milled sorghum and its starch.**	Bloomington, Ind.	Yes	3-A-2
N1 1-237	Investigation of the characteristics and classification of microorganisms of the section Dubio-rugorhizopus of the genus Rhizopus of the family Mucoraceae, as tools for use in the development of fermentations utilizing cereal crops.**	Peoria, Ill.	Yes	5-A-2
N1 1-238(C)	Studies on kernel properties and milling and fractionation characteristics of wheats exhibiting a range of kernel hardness and protein content to provide information basic to the production of a range of products for industrial uses and application in baking.**	Lincoln, Nebr.	No	
N1 1-239(Gr)	Basic investigations on the chemical and molecular structure of amyloglucosidases with emphasis on relationship to enzyme formation and action to provide information applicable to the production and use of these enzymes in the utilization of cereal grains.**	Lincoln, Nebr.	No	
N1 1-240	New microbial polysaccharides of commercial value produced from cereal grains: Characterization and structural analysis of previously selected polysaccharides and screening for additional polysaccharides with new and broader range of applicability.**	Peoria, Ill.	Yes	5-B-4
N1 1-241	Investigations on molecular structure, aggregation, and interactions of wheat gluten proteins and their chemical modifications to provide basic information related to industrial utilization of wheat.**	Peoria, Ill.	Yes	2-A-1

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-242	Chemical transformations of maltose and dextrose to determine differences in reactivity and to produce new compounds of possible industrial use from these cereal starch-derived sugars.**	Peoria, Ill.	Yes	1-A-1
N1 1-243	Modification of fermentations by transfer of genetic material in microorganisms.**	Peoria, Ill.	Yes	5-A-2
N1 1-245(C)	Development of optimal papermaking processes using cereal grain xanthides made from starch, ground whole grain, flour, bran, shorts, and other dry-milled grain products in blends with wood pulp to produce linerboard, corrugating media, and bag papers.**	Columbus, Ohio	Yes	2-B-2
N1 1-247	Exploratory studies on the chemical synthesis and characterization of crosslinked starch derivatives having potential value as paper additives for improvement of tear, stretch, and moisture stability of paper products and for upgrading properties of boxwood, insulating board, and other structural materials.**	Peoria, Ill.	No	
N1 1-248(C)	Preparation and evaluation of selected starch graft copolymers for industrial use in plastic products and industrial coatings.**	Menlo Park, Calif.	Yes	1-B-3
N1 1-249(C)	Investigations on the preparation of plastic foam from selected starch polyol derivatives and their evaluation in industrial applications.**	Minneapolis, Minn.	Yes	1-B-2
N1 1-250(Gr)	The reaction of vinyl ethers with carbohydrates, especially D-glucose and starch.**	Columbus, Ohio	Yes	1-B-4
N1 1-251(Gr)	Basic studies on the relation of viscoelastic properties of amylose sheets and films to structure and function of added plasticizers.**	Princeton, N. J.	No	
N1 1-252(Gr)	Basic investigations on the organic chemistry of unsaturated and sulfur-containing carbohydrates to provide a basis for the development of new reactions and derivatives of cereal grain starches and related sugars.**	Columbus, Ohio	No	
N1 1-253	Studies on the production of mycotoxins by Aspergillus flavus and related molds to provide basic information for processing grain into feeds.**	Peoria, Ill.	Yes	5-A-1
N1 1-254(C)	Development of improved methods for preserving microorganisms that cannot be satisfactorily lyophilized for use in the fermentative conversion of cereal grain into industrial products.**	Rockville, Md.	No	
N1 1-255	Investigation of the sporulation of milky disease bacteria in vivo and in vitro as a basis for the development of a fermentation process for the production of a pesticidal agent against the Japanese beetle.**	Peoria, Ill.	Yes	5-C-1
N1 1-256	Exploratory studies on the preparation of new and novel products from unmodified cereal starches and thin-boiling starches by graft copolymerization with selected vinyl and acrylic monomers.**	Peoria, Ill.	Yes	1-B-3

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N4 2	Soybean and other oilseed utilization investigations --Northern region.			
N4 2-72(C)	Basic physical chemical studies on linseed oil emulsions and their interaction with metal oxides.*	Los Angeles, Calif.	No	
N4 2-73	Investigations on the anionic emulsification of commercial linseed oil polymers, or simple modifications thereof, and evaluation of the emulsions as vehicles for paints.*	Peoria, Ill.	Yes	6-C-1
N4 2-74	Basic investigations on the selective hydrogenation of linolenic acid as a means of improving the flavor stability of edible soybean oil.	Peoria, Ill.	Yes	7-B-1
N4 2-77 (Rev.)	Engineering investigations on the production of cyclic fatty acids from linseed oil.	Peoria, Ill.	Yes	6-B-1
N4 2-78	Chemical and physical characterization studies on the electrophoretically and chromatographically separated proteins found in defatted soybean meal whey and on their associated nonprotein components to provide information basic to improving the industrial and feed value of whey proteins.	Peoria, Ill.	Yes	8-A-3
N4 2-79	Investigations on the major protein component of the acid-precipitated fraction of soybean proteins to gain basic information for improvement of isolated soybean protein in industrial and food products.*	Peoria, Ill.	Yes	8-A-1
N4 2-83(C)	Studies on the effect of linseed oil coatings on the durability of air-entrained concrete and its resistance to freeze-thaw cycles for evaluating the use of this oil in protecting this type of concrete against deterioration.	Manhattan, Kans.	Yes	6-C-2
N4 2-84 (Rev.)	Exploratory investigations on products obtained by reacting linseed and soybean oils and their fatty acids with selected polyols and other hydrophilic reagents, and characterization of the products for utility as water-soluble paint vehicles.	Peoria, Ill.	Yes	6-C-3
N4 2-85	Investigations on the preparation, properties, and reactions of aldehyde oils obtained by the ozonolysis of soybean, linseed and erucic acid oils, as a basis for their increased industrial utilization.	Peoria, Ill.	Yes	7-C-1,2
N4 2-86	Investigations on new polymeric products from aldehydic materials obtained by the ozonization of soybean and linseed oils, as a basis for increased industrial utilization of these oils.	Peoria, Ill.	Yes	7-C-2
N4 2-87	Engineering studies on the production of aldehyde oils from soybean, linseed, and other unsaturated vegetable oils.	Peoria, Ill.	Yes	7-C-1
N4 2-88	Basic investigations on the chemical reactions of soybean and linseed oils and their fatty acids with ethylene and other commercially available olefinic compounds to produce new products having potential industrial value.	Peoria, Ill.	Yes	6-B-1
N4 2-89	Studies on edible soybean oil: Improvements achieved by mixing soybean oil with other edible oils.	Peoria, Ill.	Yes	7-A-1,B-2
N4 2-90(C)	Investigations on flatus caused by ingestion of soybean foods as related to the development of foreign-type foods to expand export markets.	Urbana, Ill.	Yes	8-B-1

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N4 2-91	Preparation of new derivatives from soybean and linseed fatty acids or oils containing vinyl groups capable of polymerizing to form new polymers and copolymers of potential industrial value in coatings and related fields.	Peoria, Ill.	Yes	6-C-3
N4 2-92(C)	Preparation and evaluation of heterogeneous catalysts for specificity in hydrogenation of linolenate in soybean and linseed oils to increase industrial and food applications.	Chicago, Ill.	Yes	7-B-1
N4 2-93(C)	Basic investigations on heterogeneous catalysts for the selective hydrogenation of linolenate in soybean oil to provide basic information for increased food applications.	New Brunswick, N. J.	No	
N4 2-94(C)	Investigations on the conversion of aldehyde oils to coating compositions for wood and metal as a basis for developing new industrial products from linseed and soybean oils.	Fargo, N. Dak.	Yes	6-C-3
N4 2-95	Investigations on improving flavor stability of edible soybean oil by concurrent ester interchange of the oil and selective extraction of trilinolenin.	Peoria, Ill.	Yes	7-C-2
N4 2-96	Exploratory investigations on the flavor, texture, and color of soybeans, soybean fractions and products used in foods and protein supplementation to increase the use of soybeans and soybean products in foreign markets.	Peoria, Ill.	Yes	8-B-1,2
N4 2-97(C)	Basic studies on the organo complexes of transition metals as homogeneous catalysts for hydrogenation of soybean oil.	Urbana, Ill.	Yes	7-B-1
N4 2-98	Investigations on heat gelation of alcohol-washed soybean protein as a basis for developing new food and industrial uses for this protein.**	Peoria, Ill.	Yes	8-A-2
N4 2-99(C)	Studies on the effect of linseed oil coatings on the curing and durability of concrete, and evaluation of selected linseed oil compositions for this potentially new use.**	Manhattan, Kans.	No	
N4 2-100(C)	Investigations on the preparation of copolymers of linseed oil and vinyl monomers suitable for emulsion paints.**	Menlo Park, Calif.	No	
N4 2-101(C)	Investigations on the chemical and physical properties of poly(ester-acetals) and poly(amide-acetals) derived from soybean and linseed oils and of the bonds formed between them and various substrates after crosslinking.**	Dedham, Mass.	No	
N4 2-102	Microbial modification of fatty acids to produce derivative long-chain acids of potential industrial utility.**	Peoria, Ill.	No	
N5 1	Sugars and sirups investigations.			
N5 1-69	Investigations on the fermentative production of α-ketoglutaric acid from sugar or molasses to provide new industrial outlets for these agricultural materials.	Peoria, Ill.	Yes	10-A-1
N5 5	New and replacement crops utilization investigations.			
N5 5-15 (Rev.)	Chemical screening to determine the amount and kind of fiber and accompanying constituents in selected plants, as a basis for discovering potential new domestic sources of fiber for pulp and papermaking.	Peoria, Ill.	Yes	9-A-5

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N5 5-32 (Rev.)	Chemical survey of seed lipids from uncultivated domestic and foreign plants to discover sources containing economic amounts of industrially valuable constituents.	Peoria, Ill.	Yes	9-A-1
N5 5-33 (Rev.)	Characterization of selected fractions and chemical components of seeds of plant species containing favorable amounts of gross constituents to obtain more specific evaluation of their potential industrial importance than is afforded by screening analyses.	Peoria, Ill.	Yes	9-A-2.B-2
N5 5-40(C)	Chemical survey of native, introduced, or newly developed strains of Brassica and related genera of mustard seed to find seed oils with maximum erucic acid content, as a basis for development of new industrial oils from domestic crop sources.*	Bozeman, Mont.	Yes	9-A-1
N5 5-41	Investigation of selected plants of the Hibiscus genus, with emphasis on kenaf and okra, to evaluate and develop fibrous products from annual plant sources having superiority or specific preferred properties for industrial use.	Peoria, Ill.	Yes	9-C-1
N5 5-44	Analytical investigations on proteins and other nitrogenous substances in meals from potential new oilseed crops, with emphasis on those bearing oils of high erucic acid content, to obtain fundamental information of value in their processing and utilization.	Peoria, Ill.	Yes	9-A-3
N5 5-47	Engineering studies on a process for converting Crambe abyssinica seed and closely related new oilseeds into oil and detoxified meal for evaluating the utilization potential of these new oilseed crops.	Peoria, Ill.	Yes	9-B-1
N5 5-48(C)	Investigations on the preparation of omega-amino tridecanoic acid from crambe oil, the production of polyamides from it, and evaluation of the polymers for industrial uses.**	Birmingham, Ala.	No	
NU P-1	Pioneering Laboratory for Microbiological Chemistry.	Peoria, Ill.	Yes	5-A-3

*Discontinued during reporting year.
**Initiated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
(10)	Cereal and forage crops.			
UR-A7-(10)-7	Fundamental studies of enzyme systems isolated from Pseudomonas, to obtain information on the conversion of carbohydrates derived from cereal grains to organic acids having potential industrial value.	Bangalore, India	No	
UR-A7-(10)-9	Collection and isolation of molds belonging to the order Mucorales, and classification of the isolates, in order to find microorganisms suitable for fermentative processes of importance in cereal grain utilization.	Allahabad, India	Yes	5-A-1
UR-A7-(10)-10	A study of survival and possible genetic change in industrially useful microorganisms subjected to lyophilization, to obtain basic information needed for the maintenance of culture collections for industrial fermentation of cereal grains.	Allahabad, India	Yes	5-A-1
UR-A7-(10)-20	Investigations on the preparation and characterization of new copolymers of cereal starch with other polysaccharides by heating mixtures in the dry state, to provide basic information for the development of new starch products suited for industrial applications.	Ahmedabad, India	Yes	1-B-4
UR-A7-(10)-25	Investigations on the separation of grain sorghum proteins into homogeneous protein components, to provide basic information for further characterization and application studies.	Bangalore, India	Yes	3-A-4
UR-A10-(10)-1	A fundamental investigation of the synthesis and chemical and physical properties of multichain polymers and copolymers comprised of amino acids derivable from the cereal grain protein, gliadin and zein, as a contribution to the increased utilization of cereal grains.*	Rehovot, Israel	Yes	2,3-A-3
UR-A10-(10)-9	Studies of the preparation of new cereal starch derivatives by the introduction of fluorine into starch and products derived therefrom, to provide a basis for the increased industrial utilization of wheat, corn, and sorghum.*	Jerusalem, Israel	Yes	1-B-4
UR-A10-(10)-27	Studies on the preparation and properties of graft copolymers of starch and dextrin obtained by reaction with vinyl monomers and epoxides, to provide a basis for increased industrial utilization of cereal grains.**	Jerusalem, Israel	Yes	1-B-3
UR-A10-(10)-51	Fundamental studies on the mild oxidation of cereal grain starches by selected oxidizing agents for the determination of reaction mechanisms and the physical and chemical properties of modified starches of importance to their production and industrial use.**	Jerusalem, Israel	No	
UR-E8-(10)-6	Isolation of organic phosphorus derivatives found in the yeast Torulopsis utilis and elucidation of their structures, to provide new basic information on the fermentation of cereal products to industrial materials by yeasts.	Helsinki, Finland	Yes	5-A-3

*Completed project.
**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-E9-(10)-37	Fundamental investigations of the proteolysis-inhibiting effects of cereal flours and starches, and of processing methods for minimizing such effects, to provide a basis for improved quality and increased utilization of cereal products.	Paris, France	Yes	1-A-6
UR-E9-(10)-40	Investigations of the zein protein of corn: Fractionation and study of rheological and physical-chemical properties, chemical composition and structure, and problems of hydration and gelification of fundamental importance to the technology and industrial utilization of corn proteins.	Paris, France	Yes	3-A-4
UR-E9-(10)-42	A fundamental investigation of the physico-chemical alterations brought about in starches and their molecular constituents by gamma-radiations, to provide information needed for modification of starch properties and for the treatment of starch-containing products used industrially or in foods.	Paris, France	Yes	1-A-7
UR-E9-(10)-56	Selection and mutation of strains of yeast capable of producing high quantities of sulfur-containing amino acids for use in increasing the efficiency of cereal-grain-based feeds deficient in these amino acids.**	Paris, France	No	
UR-E15-(10)-21	Investigation of the growth factor (Vitamin B_{13}) of distillers' dried solubles through studies of methods of isolation and purification, mode of formation, and conditions of optimum production by yeast fermentation of cereal grains, to provide basic information for utilizing grains to produce this vitamin.	Milan, Italy	Yes	5-D-3
UR-E15-(10)-24	Investigation of aerobic fermentation processes by measurement of the effects of differences in vessel size and mechanical agitation on the concentration of dissolved oxygen, and by studies of the physiochemical properties of the foam, to obtain fundamental information needed for the increased utilization of cereal grains in fermentative processes.*	Rome, Italy	Yes	5-B-6
UR-E15-(10)-25	Investigations of the reaction of cereal starch dextrins with fatty acid chlorides and fatty amines, and evaluation of the products, to provide information important to increasing the utilization of wheat, corn, and sorghum.	Bologna, Italy	Yes	1-B-4
UR-E15-(10)-26	Investigation of the fermentative conversion of glucose to 5-ketogluconic acid through studies of a metabolic pathway in organisms of the Acetobacter genus, to obtain fundamental information for the utilization of grain products in the fermentative production of chemical intermediates.	Milan, Italy	Yes	5-A-3
UR-E15-(10)-32	Investigations on the conformation of gluco-pyranose rings in amylose corn starches and in linear and cyclic dextrins prepared from these starches, to provide basic information for the chemical modification of starch-derived products for the development of new uses.	Milan, Italy	Yes	1-A-5

*Completed project.
**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
UR-E21-(10)-11	Investigations on the fermentative production of itatartaric acid from glucose, sucrose, or molasses to provide new industrial outlets for these agricultural materials.**	Lodz, Poland	Yes	5-B-5
UR-E25-(10)-11	Isolation and characterization of yeasts for placement in the Culture Collection of the Agricultural Research Service, as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.	Madrid, Spain	Yes	5-A-1
UR-E29-(10)-36	Fundamental studies of chemical reactions for polymerizing glucose and glucose derivatives to form new high-molecular-weight compounds, as a basis for the development of new outlets for cereal grains and other starch-rich crops.*	Musselburgh, Scotland	Yes	1-B-7
UR-E29-(10)-37	Studies on the quantitative measurement of properties of wheat kernels that vary significantly during conditioning, as a basis for improved conditioning of wheat for milling by new and improved methods and increased industrial utilization of flour and milled wheat products.	St. Albans, England	Yes	2-C-6
UR-E29-(10)-39	A fundamental study of factors governing the onset of oxidative rancidity in oat products, to provide a basis for improving the quality and increasing the utilization of oats in feed and food.	St. Albans, England	Yes	3-A-5
UR-E29-(10)-40	Investigations of the structure and properties of cereal starches--particularly corn and wheat starches--as revealed by their interaction with enzymes and other proteins, to obtain fundamental information concerning the structure and behavior of cereal starches that would be useful in starch processing.	Edgbaston, Birmingham, England	Yes	1-A-5
UR-E29-(10)-51	Investigation of sugars, their phosphate derivatives, and related compounds, as found in molds important to the fermentative conversion of cereal grains to useful products.	Newcastle-upon-Tyne, England	Yes	5-A-3
UR-E29-(10)-69	Fundamental studies on the nature and specificity of starch- and glycogen-debranching enzymes and the application of these enzymes to a study of the fine structures of amylopectins, amyloses, and glycogens of cereal grains, to provide a basis for increased utilization of cereal grains.**	London, England	Yes	1-A-5
UR-E30-(10)-1	Studies on the modification of cereal grain starches by physical treatment of granular starch under different conditions of moisture, temperature, and pressure in order to impart new paste properties as a basis for increased utilization of cereal grains.**	Ljubljana, Yugoslavia	No	
UR-S3-(10)-11	Preparation of cationic cereal starch derivatives for use in paper and textiles by the introduction of quaternary phosphonium and tertiary sulfonium groups into crosslinked and noncrosslinked starches, to create new markets and expand old markets for starch from cereal grains.	Rio de Janeiro, Brazil	Yes	1-B-4

*Completed project.
**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
(10,40)	Cereal, forage crops, and oilseeds.			
UR-A11-(10,40)-10	Investigation of crosses of Saccharomyces rouxii isolated from the soybean fermentations, shoyu and miso, and an evaluation of their fermentative abilities in the above fermentation processes, as a basis for increasing the use of soybeans in fermented foods.	Noda-shi, Chiba-ken, Japan	Yes	8-B-4
(40)	Oilseeds			
UR-A6-(40)-1	Investigation of the various processes used in preparing Chinese cheese by the fermentation of soybean curd with Mucor and other fungi as a basis for increasing the foreign utilization of soybeans.	Taipei, Taiwan	Yes	8-B-6
UR-A7-(40)-21	Exploratory investigations of selected hydroxylated derivatives of linseed and safflower oils, to determine the feasibility of producing new industrial products from these oils.	Hyderabad, India	Yes	6-B-3
UR-A10-(40)-17	Fundamental investigations of complexes formed by soybean proteins with other meal constituents, to provide information for applied studies on expanded utilization of soybean oil meal.	Rehovot, Israel	Yes	8-A-5
UR-A10-(40)-18	Investigations of soybean saponins as related to the processing of petroleum ether-extracted meal for feed and to the preparation of soy foods, to provide information basic to improving the nutritional value of soybean protein products.	Rehovot, Israel	Yes	8-C-1
UR-A10-(40)-20	Laboratory investigations on miso-type food products by fermentation of soybean meal products and cereal grains for use in Israeli foods.	Ramat Gan, Israel	Yes	8-B-4
UR-A10-(40)-30	Investigations of the effect of processing conditions on the yield and quality of isolated soybean protein for use in Israeli-type foods, as a contribution to expanded utilization of soybeans.	Haifa, Israel	Yes	8-B-5
UR-A11-(40)-1(C)	Factory experiments on comparative production of shoyu (soy sauce) from United States and Japanese soybeans, to provide data for the increased use of United States beans.*	Tokyo, Japan	Yes	8-B-3
UR-A11-(40)-2	Evaluation of dehulled soybean grits from United States varieties for making miso, to increase soybean utilization in Japan.	Tokyo, Japan	Yes	8-B-4
UR-A11-(40)-5	Investigation of the partial hydrogenation of soybean oil, to produce a stable liquid oil with improved properties for use in Japanese foods.	Kawagoe, Saitama-ken, Japan	Yes	7-B-4
UR-A11-(40)-11	Evaluation of United States soybean varieties as a material for producing fresh tofu to increase utilization in Japan.	Tokyo, Japan	Yes	8-B-4
UR-A11-(40)-12	A chromatographic study of the sugars and oligosaccharides in soybeans to provide information needed to improve processing of fat-free soybean meal for foods and feeds, thereby contributing to its expanded utilization.	Takamatsu, Japan	No	

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PL 480 Research Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
UR-A11-(40)-13	Isolation, characterization, and quantitative determination of the sterols in soybeans to provide basic information for the evaluation and improvement of soybean meal and soybean products as foods and feeds.**	Tokyo, Japan	Yes	8-A-6
UR-E8-(40)-2	Investigation of continuous multi-stage countercurrent crystallization of linseed and soybean fatty acids as a practical method for producing pure unsaturated fatty acids, to provide a basis for new or improved uses of linseed and soybean oils.	Helsinki, Finland	Yes	7-C-3
UR-E15-(40)-8	An investigation of the minor constituents of linseed oil and their effect on the ability of linseed oil films to spread and adhere to surfaces, as a contribution to the expansion of markets for linseed oil.	Milan, Italy	Yes	6-A-1
UR-E15-(40)-9	Investigations of the controlled thermal polymerization of soybean and linseed oils, and of the separation and characterization of the reaction products, in order to obtain information useful in expanding and improving the industrial applicability of these oils.	Milan, Italy	Yes	6-B-2
UR-E15-(40)-10	Investigations of the effect of metallic catalysts and physical conditions on oxidative cleavage products produced in the autoxidation of polyunsaturated fatty acids, to provide basic information for applied research on the production of new industrial chemicals from soybean and linseed oils.	Milan, Italy	No	
UR-E15-(40)-14	Studies of the admixture of soybean protein products with wheat flour in the manufacture of pasta (spaghetti, macaroni, etc.) to effect improvements in diets largely based upon cereals and contribute to increased utilization of soybeans.***	Rome, Italy	No	
UR-E21-(40)-6	Chromatographic determination of the glyceride composition of selected erucic-acid containing oils, to provide basic information important to their utilization.	Warsaw, Poland	Yes	9-A-2
UR-E21-(40)-8	Investigation of the possible role of sterols in the development of flavors and odors in soybean oil through studies of sterol transformations during processing, in order to increase the utilization of soybeans in food.	Gdansk, Poland	Yes	7-A-3
UR-E25-(40)-4	Investigations of ion exchange procedures for removing pro-oxidant metals from soybean oil, in order to contribute to expanded utilization of soybean oil through improvement of its flavor and oxidative stability during transportation, storage, and use.	Seville, Spain	Yes	7-A-2
UR-E25-(40)-29	Improvement of the frying quality of soybean oil through studies of the influence of processing factors and oil modifications on surface tension, interfacial tension, viscosity, and other physical properties concerned with its penetration into fried foods, to provide information for increased use in the preparation of Spanish foods.	Granada, Spain	Yes	7-B-3

**Activated during reporting year.
***Cancelled.

PL 480 Research Project Check List -- Reporting Year July 1, 1963 to June 30, 1964 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-E26-(40)-3	Compositional investigations of Swedish Cruciferae (mustard family) seeds to find strains with maximum erucic acid content in their oils and minimum content of glucosidic precursors of isothiocyanates and thiooxazolidones in their meals, to provide a basis for their utilization as industrial oilseeds in the United States.**	Svalof, Sweden	No	
UR-E29-(40)-29	Development of new uses for soybean and linseed oils through investigations of organometallic derivatives and complexes as components of protective coatings having improved properties.	Teddington, Middlesex, England	Yes	6-C-4
UR-E29-(40)-49	Investigation of the reactions of unsaturated fatty acids and their derivatives in molten alkali, to discover new chemical intermediates important to the increased utilization of soybean and linseed oils.	London, England	Yes	7-C-4
UR-E29-(40)-50	A quantitative study of the polysaccharides in fat-free soybean meal to provide information needed to improve the processing of meal for foods and feeds, thereby contributing to its expanded utilization.	Edinburgh, Scotland	Yes	8-A-4
(50)	Sugar and miscellaneous crops.			
UR-E15-(50)-29	Preparation and characterization of dextran derivatives, and investigations of their interactions and binding, to provide basic information for increasing the utilization of sugar.	Rome, Italy	Yes	10-A-2

**Activated during reporting year.